Report on Bioavailability of Chemical Wastes With Respect to the Potential for Soil Bioremediation

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Contents

EXECUTIVE SUMMARY

Based on conservative, reasonably thorough and careful evaluation of scientific studies described in this report, there is no doubt that chemical wastes in soil can be, and often are, in a state of reduced bioavailability. An analysis of the literature on bioremediation research concludes that bioremediation of chemical wastes in soils and sediments is rarely 100 percent efficient, due at least in part to the reduced bioavailability of the chemical. Reduced bioavailability simply means that a chemical waste's diminished "effective concentration" is proportionately balanced by a lingering reservoir of the chemical waste in soil and sediments. This lingering reservoir remains in the soil habitat regardless of which combinations of conceptual or actual sequestration mechanisms (e.g., complexation into bound residues, diffusion into soil pores, NAPL partitioning) apply.

Soil is, by definition, a thermodynamically unstable, kinetically constrained medium whose chemical composition, (solid, liquid, and gaseous phases) is constantly changing. Thus, the "nonbioavailable" chemical wastes in this lingering reservoir are always subject to release into soil solution where the wastes are resubjected to a variety of transport and/or transformation processes (e.g., immobilization, biodegradation, uptake by receptors).

Considerable effort has been expended in investigating the hypothesis that chemical wastes have diminished bioavailability in soils and sediments. Results of these efforts have been ambiguous because of the immense diversity in types and properties of chemical wastes, geosorbents, biota, experimental approaches, and the idiosyncrasies in mechanisms by which biota interact with chemical wastes. In this report, 17 studies were selected from the vast literature on bioavailability to illustrate the range in quality of reported research. Methodologies were analyzed, and conclusions were critiqued. From this analysis, criteria for identifying the highest quality investigations pertinent to bioavailability are proposed. These are based on the realism and environmental relevance of the study, the absence of experimental artifacts, and the consistency of the results.

An accurate estimate of risks to human and environmental health posed by chemical wastes in soils is a crucial step toward: (1) identifying pragmatic, economically feasible environmental cleanup goals; (2) establishing operational definitions of "treatment" by bioremediation technology; (3) realistically classifying

polluted sites based on planned land-use scenarios; (4) developing public acceptance of risk-based contaminant cleanup efforts; (5) developing public acceptance of cleanup goals that are above the "original, pristine state" of the contaminated site; and (6) legitimizing the concept of "environmentally acceptable endpoints."

From a practical, regulatory point of view, establishing the foundation of reduced bioavailability is crucial. If the reduced bioavailability of chemical wastes in soil becomes widely accepted, then proper quantitative measures of bioavailability reduction could be developed to accurately estimate the risks posed by chemical wastes in soils and sediments to human health and ecological processes.

Section 1

Introduction: Scope and Goals

Commercial, industrial, and military activity, largely in the 19th and 20th centuries, have led to environmental contamination problems that can threaten human health and ecosystem function (Alexander, 1999; Madsen, 1998b; National Research Council, 1997; Young and Cerniglia, 1995). The degree to which such contaminants are available to biota has far-reaching scientific, toxicological, and regulatory implications (e.g., Lee *et al*., 2000). Bioremediation, the use of organisms (especially naturally occurring microorganisms or plants) to eliminate these pollution problems has immense potential for meeting society's pollution-control needs (Alexander, 1999; Madsen, 1998b; National Research Council, 1993, 1997; Schwarzenbach *et al*., 1999; Van der Lelie *et al*., 2001; Young and Cerniglia, 1995). However, in recent years, evidence has begun to accumulate suggesting that pollution-eliminating processes may be thwarted by a variety of physical, chemical, or other spontaneous reactions that diminish the availability of environmental contaminants for uptake and transformation by biota (Alexander, 1995, 1999).

This report critically evaluates current knowledge of the relationships between the bioavailability of chemical wastes and their susceptibility to bioremediation. The context of these relationships is that all terrestrial and aquatic habitats are located in either the surface or subsurface portions of the landscape. However, due to this report's focus, soil habitats will be emphasized. Although model systems using laboratory incubations of environmental samples or cultures of individual microorganisms provide insights about these relationships, **the primary focus of this report is the behavior of chemical wastes and naturally occurring (or inoculated) microorganisms *in situ* — in real-world contaminated field sites**. When appropriate, information about the nascent field of phytoremediation (plant-mediated decontamination processes) will be included. The chemical wastes of interest are those that confront society today. These include organic (e.g., petroleum hydrocarbons, pesticides, chlorinated solvents) and inorganic (e.g., metals, radionuclides, oxyanions) compounds. This report will not attempt to comprehensively

survey the behavior of all contaminants. Instead, compounds will be selected from both categories (organic and inorganic) to reveal how microorganisms and plants interact with chemical wastes. There is no doubt that, **under favorable conditions**, many contaminants and microorganisms or plants can interact in ways that successfully lead to biodegradation and bioremediation. The issue addressed by this report is how bioavailability influences the degree to which the bioremediation outcome is successful (according to the standards of regulatory agencies and society).

This report is divided into 10 sections, including this introduction. Section 2 will begin by defining biodegradation and bioremediation; because of their novelty, fundamental microbial reactions that govern the fate of inorganic materials are reviewed in detail. Section 3 defines bioavailability. Next, selected case studies of completed bioremediation field projects are presented—seeking evidence for a reduction in the efficacy of organic- and inorganic-contaminant bioremediation that may have been caused by bioavailability limitations. To interpret how and why bioremediation may be inefficient, Section 5 reviews information about the mechanisms by which organic compounds persist in soils. In Section 6, paradigms for geosorbents and the physical and chemical state of associated contaminants in field sites will be summarized. Section 7 reviews physiological principles describing the uptake of sequestered organic and inorganic chemicals by microorganisms and plants. Assisted by recent scientific reviews, Section 8 critically evaluates the methodologies that have been used to generate information about the influence of bioavailability on bioremediation. Section 9 examines studies designed to enhance the efficiency of remediating organic compounds in soil by alleviating bioavailability limitations. The report concludes (Section 10) with recommendations about new frontiers and areas of future research.

This document is heuristic—it strives to synthesize new combinations of information of the highest quality from a variety of sources. The sponsor of this report (the US EPA) prescribed that it be a concise compilation of current knowledge, not an encyclopedic review. In compiling and processing information for this report, difficult decisions were made to include some while excluding other scientific studies. The author apologizes for any omissions of crucial pertinent scientific results. **Throughout this report, selected portions of the text will be highlighted in bold because they figure prominently in the logic that will lead to this report's conclusions**.

Section 2

Definitions of Biodegradation and Bioremediation, and the Many Facets of Bioavailability

2.1 Biodegradation

"Biodegradation" is the partial simplification or complete destruction of the molecular structure of environmental pollutants by complex, genetically regulated physiological reactions catalyzed largely by microorganisms (Alexander, 1999; Madsen, 1991; Madsen, 1998b; Young and Cerniglia, 1995); and plants (Bhadra *et al*., 1999; Bizily *et al*., 1999; Burken and Schnoor, 1998; Siciliano and Germida, 1998). Many of these reactions are predictable based on established laws of thermodynamics applied under environmental geochemical conditions (e.g., temperature, pressure, and oxidation-reduction potential) that prevail in terrestrial and aquatic habitats (Madsen, 1998a). Microbial biodegradation is routinely measured by applying chemical and physiological assays to laboratory incubations of flasks containing pure cultures of microorganisms, mixed cultures, or environmental samples (soil, water, or sediment; Madsen, 2002). Plant-mediated biodegradation and/or contaminant accumulation also is routinely documented in appropriately scaled laboratory test systems (Bhadra *et al*., 1999; Bizily *et al*., 1999; Van der Lelie *et al*., 2001; Vangronsveld and Cunningham, 1998).

2.2 Bioremediation

"Bioremediation" is the intentional use of biodegradation or contaminant-accumulation processes to eliminate environmental pollutants from sites where they have been released. Bioremediation technologies use the physiological potential of microorganisms and plants, as documented most readily in laboratory assays, to eliminate or reduce the concentration of environmental pollutants in field

sites to levels that are acceptable to site owners and/or regulatory agencies (Burken and Schnoor, 1998; Madsen, 1998b; National Research Council, 1997; Siciliano and Germida, 1998). Bioremediation may be approached using *in situ* technology (applied directly to contaminated sites) or *ex situ* bioreactor methodologies (after contaminants and/or accompanying soil, sediment, or water are removed from contaminated sites) (Crawford and Crawford, 1996; Flathman *et al*., 1994; Hinchee and Olfenbuttel, 1994; Madsen, 1998b; Norris *et al*., 1994; Rittman *et al*., 1994; Skipper and Turco, 1995; Siciliano and Germida, 1998). The open, complex, poorly defined settings where bioremediation occurs and the often wide array of possible contaminant attenuation pathways may obscure the precise mechanisms that operate during field bioremediation operations (Madsen, 1991; National Research Council, 1993; National Research Council, 2000).

Phytoremediation is a technology that seeks to use several plant-based processes to remove, transfer, stabilize, or destroy contaminants in soil, sediment, and groundwater (Cunningham *et al*., 1997; Ensley, 2000; EPA, 2001; Van der Lelie *et al*., 2001; Vangronsveld and Cunningham, 1998). The mechanisms of phytoremediation include rhizosphere biodegradation, phytoextraction, phytodegradation, and phytostabilization. Phytoremediation strategies can use long-lived perennial species in a stable system or short-lived annual species that are repeatedly planted and harvested.

2.3 The Susceptibility of Inorganic Materials, Including Metals, Nonmetals, and Radionucleotides, to Microbe-Based Bioremediation

Biological processes can influence inorganic environmental contaminants (Babu *et al*., 1992; Brierley, 1990; Chapatwala *et al*., 1995; Hinchee *et al*., 1995; Kalin et al., 1991; Lenhard et al., 1995; Lovley, 1993, 1995a, b; Lovley, 2000; McHale and McHale, 1994; Saouter et al., 1995; Summers, 1992; Thompson-Eagle and Frankenberger, 1992; Videla and Characklis, 1992; and Whitlock, 1990). Unlike organic compounds that are often susceptible to partial structural alteration or complete detoxification to carbon dioxide by microorganisms, the majority of inorganic contaminant compounds are subject only to changes in speciation that may influence contaminant mobility. Microorganisms or plants may cause precipitation, volatilization, sorption, and solubilization of inorganic compounds. Mechanistically, these reactions can be the result of direct enzymatic processes such as oxidation, reduction, methylation, or uptake. Reaction mechanisms also can be indirect (nonenzymatic)—resulting from production of metabolites or biomass that can strongly influence the behavior of inorganic contaminants via redox, acid/base, coprecipitation, sorption, and other geochemical means. In many phytoremediation scenarios, contaminant-attenuation mechanisms are driven by transpiration-based hydrodynamic interception of contaminants in soil solution and groundwater. Direct physiological transformation of inorganic com-

pounds by plants is an area of current research, as is plant-microbe interactions in the rhizosphere. Because the science and technology of microbial processes that influence inorganic compounds are well developed, microbial-, and not plant-based, bioremediation is emphasized below. However, some microbial reaction types may apply to plants, and certainly apply to the rhizosphere.

One nearly universal means by which microorganisms have been shown to reduce concentrations of inorganic contaminants in water (e.g., Cu, Ni, Zn, Cd, Pb) is by immobilizing aqueous-phase inorganics in microbial biomass and/or microbial exopolymers (Diels, 1997; Macaskie and Basnakova, 1998). The mechanisms range from nonspecific electrostatic sorptive interaction between cationic metals and anionic extracellular polysaccharides (Williams et al., 1998) to highly specific active transport systems that cause metals to accumulate in high concentrations within microbial cells (Chen and Wilson, 1997). The utility of these sequestration reactions for soil is in doubt but they are promising in engineered wastewater treatment systems where metal-laden water flows over fixed biofilms that can be removed from the treatment system so that the toxic inorganics can be recovered.

Many inorganic contaminants, especially metals, become relatively soluble or mobile at low pH. In contrast to the various bioremediation approaches that rely on immobilization reactions, the opposite (washing soils and sediments free of inorganic contaminants) can theoretically be achieved by directing low pH waters through contaminated sites. The acidification step can be mediated by a variety of microbial processes that include the oxidation of elemental sulfur (Lovley and Coates, 1997).

The often highly abundant nontoxic metals, iron and manganese, exist in reduced and oxidized states. The oxidized states [Fe(III), Mn(IV)] react chemically to form oxyhydroxide precipitates that serve as physiological electron acceptors for anaerobic microbial food chains (Lovley, 1995a; 2000). The end products of Fe- and Mn-reduction [Fe(II) and Mn(II)] are relatively soluble and may migrate to aerobic habitats, where reoxidation and precipitation also can be catalyzed by microorganisms (Ghiorse, 1994). The behavior of many of the toxic metals discussed below is intimately tied to the microbially mediated cycling of Fe and Mn because the toxic metals may be immobilized (through coprecipitation and sorptive reactions with many Fe and Mn oxides) or solubilized [by being reduced via chemical reactions with Fe(II) and Mn(II)] (Martinez and McBride, 2001). Thus, most problem inorganic compounds (e.g., Pb, Cr, U, Ni, Hg, Cd, Sr) undergo immobilization reactions via sorption and precipitation.

Chromium is a metal whose key oxidation states are (VI) and (III) (NRC, 2000). In aqueous environments, chromium (VI) predominates as the mobile and highly toxic anions, chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). Reduced chromium (III) is less toxic and less mobile because of precipitation reactions as oxides and hydroxides at pH 5 and above. A variety of both aerobic and anaerobic microorganisms have been shown to enzymatically reduce Cr(VI) to Cr(III), but the physiological reason for this

ability has not been adequately investigated. Among the hypotheses explaining the reduction reactions are: survival (i.e., detoxification), cometabolism (i.e., fortuitous enzymatic reactions), and the use of Cr(VI) as a physiological electron acceptor (to date, only equivocal evidence for the latter hypothesis has been obtained). Direct microbial detoxification (reduction) of Cr(VI) is unlikely to be a useful remediation technology in anaerobic subsurface habitats because the reduction occurs spontaneously in the presence of sulfide, Fe(II), and some organic compounds. Although microbial production of sulfide, Fe(II), and reduced organic compounds is generally reliable, additional research is required before judging if Cr(VI) reduction has the potential to serve as a useful bioremediation tool (NRC, 2000).

Mercury is a toxic metal whose predominant forms include mercuric ion [Hg(II)], elemental mercury [Hg(0)], and the biomagnification-prone organic mercury compounds, monomethyl- and dimethyl-mercury (NRC, 2000). All transformations of mercury by microorganisms are considered detoxification reactions that are intended to mobilize mercury away from microbial cells. Most reactions are enzymatic, carried out by both aerobes and anaerobes, and involve uptake of Hg(II) followed by its reduction to volatile forms (elemental Hg(0), methyl-, and dimethylmercury). Mercuric ion [Hg(II)] also forms highly insoluble precipitates with sulfide; thus, one indirect microbial detoxification strategy involves the stimulation of sulfate-reducing microorganisms. Although engineered bioreactors that first reduce the mercuric ions and then purge the volatile Hg from water have been designed, no successful applications of this technology have yet been implemented in soil environments (NRC, 2000).

In addition to mercury, microorganisms are capable of methylating other metals (Cd, Pb, Sn, Te, Se; Ehrlich, 1996; Se methylation is discussed in detail below). Additional methylation reactions may occur as a result of nonbiological transmethylation by microbially produced methylated donor compounds such as trimethyl tin (Ehrlich, 1996). These donors may react with ionic forms of Pd, Th, Pt, and Au, but the resultant reduced metals may not be chemically stable. The significance of these unusual metal methylation reactions for bioremediation is unknown.

Arsenic is a toxic element capable of existing in five different valence states [As (-III), (0), (II), (III), and (V)]. Forms of arsenic range from sulfide minerals (e.g., As_2S_3) to elemental As to arsenic acid to arsenite (AsO_2^-) to arsenate (AsO_4^{3-}) to various organic forms that include methylated arsenates and trimethyl arsine. Clearly, the chemical and microbiological reactions of As are complex (Ehrlich, 1996; Frankenberger and Losi, 1995). Both anionic forms (arsenite and arsenate) are highly soluble and highly toxic—interfering with various enzyme functions and oxidative phosphorylation, respectively. All forms of As are toxic. Microorganisms transform arsenic for several fundamental physiological reasons: (1) under anaerobic conditions, arsenate [As(V)] can be used as a final electron acceptor; (2) under aerobic conditions, reduced As (e.g., arsenite) oxidation had been shown to generate energy (ATP); and (3) under both anaerobic and aerobic conditions, As can be detoxi-

fied by methylation, oxidation, or reduction mechanisms that mobilize As away from microbial cells. Engineered bioremediation strategies that rely on mobilizing methylated As from water have been implemented (Frankenberger and Losi, 1995).

Although selenium is an important and beneficial micronutrient for plants, animals, humans, and some microorganisms (largely because of its role in some key amino acids), this element can be toxic at greater than trace concentrations. In natural environments, selenium has four predominant inorganic species: Se(VI) (selenate, SeO_4^{2-}), Se(IV) (selenite, SeO_3^{2-}), Se(0) (elemental selenium), and Se(-II) (selenide) (Ehrlich, 1996; Lovley, 1995b; Frankenberger and Losi, 1995). Like arsenic, selenium also has many volatile organic forms; these include: dimethyl selenide, dimethyl diselenide, methane selanone, methane selenol, and dimethyl selenyl sulfide. Each of these compounds exhibits its own chemical and biochemical behavior, mobility, and toxicity. The various forms of selenium are transformed by microorganisms according to their physiological needs and the ambient thermodynamic conditions. Reduced inorganic selenium compounds have been shown to be oxidized under aerobic conditions, though not linked to microbial growth. Oxidized selenium (selenate) can serve as a final electron acceptor for anaerobic microorganisms—resulting in production of selenide and/or elemental Se. Methylation of the various Se compounds is thought to be a protective detoxification mechanism that mobilizes Se away from microbial cells. Thus like As, the environmental fate of Se is governed by complex interactions between chemical and physiological processes. For instance, anaerobic microbial reduction of selenate and selenite to insoluble elemental selenium represents an important mechanism for immobilizing and removing Se from aqueous solution. Furthermore, the various volatile methylated forms of Se are sufficiently mobile that aerobic deselenification (largely via dimethylselenide formation) of highly contaminated California soils has been demonstrated in field experiments (Frankenberger and Losi, 1995).

Oxyanions are water soluble, negatively charged chemical species in which a central atom is surrounded by oxygen. Nitrate (NO_3^-) is a naturally occurring oxyanion commonly found at low concentrations in soils, sediments, and both surface and groundwaters as a result of the biogeochemical cycling of organic matter. Nitrate is a readily utilizable form of nitrogen that can be assimilated into amino acids by plants and microorganisms (NRC, 2000). Although serving to supply nitrogen, an essential nutrient, nitrate also is a serious health concern for at least two reasons: (1) it can be a chemical or microbiological precursor for nitrite, which spontaneously combines with amino compounds to form highly carcinogenic nitrosamines; and (2) nitrate can be reduced to nitrite in stomachs of infants, which can cause the respiratory stress disease, methemaglobinemia.

Nitrate is produced from ammonia by nitrifying microorganisms under aerobic conditions. The major microbial process that destroys nitrate is dissimilatory reduction to dinitrogen gas (denitrification). Genetic, biochemical, physiological, ecological, environmental**,** and engineering aspects of denitrification (Zumft, 1997; Atlas and

Bartha, 1997) have been examined for decades. Nitrate is used as a physiological electron acceptor under oxygen-free (anaerobic conditions). The denitrification process is widespread among microorganisms, and occurs reliably in every anaerobic habitat with abundant carbon and electron sources. Denitrification may not be applicable to surface soil because of the presence of O_2 and the tendency of nitrate to leach. However, denitrification is a well-established bioremediation process and is used routinely in sewage treatment plants to curb eutrophication.

The oxyanions chlorate (ClO_3^-) and perchlorate (ClO_4^-) or their precursors (chlorine dioxide, hypochlorite, and chlorite) are produced by a variety of paper manufacturing, water disinfection, and both aerospace and defense industries. Although not naturally occurring, these highly oxidized forms of chlorine are energetically very favorable physiological electron acceptors for microorganisms. Compared to denitrification, knowledge of chlorate and perchlorate biodegradation reactions is quite limited. However, laboratory studies using both pure bacterial cultures and environmental samples (soil, freshwater sediments, and sewage) have shown that in the presence of common electron donors (carbohydrates, carboxylic acids, amino acids, even H_2 and H_2S), microorganisms can grow at the expense of perchlorate and chlorate; thus reducing them to the nontoxic chloride ion (Malmqvist et al., 1991; vanGinkel et al., 1995; Rikkon et al., 1996). Furthermore, a bioreactor has recently been engineered to successfully convert chlorate and perchlorate to chloride (Wallace et al,. 1997).

Uranium is a radioactive element that releases alpha, beta, and gamma radiation that can be toxic. Uranium can exist in the oxidation states of (0), (III), (IV), (V), and (VI), though in nature insoluble $U(IV)O_2$ and soluble $U(VI)O_2^{2+}$ predominate. Recently, U(VI) has been shown to serve as a terminal electron acceptor for anaerobic microorganisms; thus, in anaerobic habitats growth-linked reduction (hence immobilization) of U should be a reliable process (Lovley, 1995a). Although the reverse process, microbial oxidation of U(IV) to U(VI) under aerobic conditions has been demonstrated, this process has not been shown to be physiologically beneficial to the responsible microorganisms. Direct chemical oxidation of U(IV) by molecular oxygen [creating U(VI)] may also influence the robustness of U bioremediation strategies (NRC, 2000). Another radioactive element, plutonium (Pu) also has been shown to be susceptible to microbial transformation. Iron-reducing microorganisms were found to reduce insoluble Pu^{4+} to the more soluble Pu^{3+}; thus, rendering the soluble form more susceptible to mobilization (NRC, 2000). These microbially mediated oxidation/reduction reactions provide tools for emerging bioremediation strategies for U, Pu, and other radionuclides.

2.4 Summary

Biodegradation is a complex series of metabolic processes that simplify the molecular structure of organic compounds. Biodegradation is catalyzed largely by microorganisms. Plants also can cause biodegradation reactions, but they are more suited for uptake and accumulation reactions. Inorganic contaminants (metals, non-metals,

oxyanions, and radionuclides) cannot be biodegraded, but their environmental mobility can be altered through oxidation-reduction, sorption, methylation and precipitation reactions mediated by microorganisms or plants. Each inorganic pollutant features a unique set of direct and indirect biotic and abiotic reaction pathways that may be exploitable by bioremediation technologies. Biodegradation, accumulation, and an altered mobility of contaminants can be rigorously documented in laboratory experiments. These processes are the basis for potential site cleanup technology.

Section 3

Bioavailability

3.1 Defining Bioavailability: Nine Definitions

Microorganisms and plants are the catalysts that effect bioremediation. But, as discussed in Section 1.0, the "bioavailability" of chemical waste in soils, sediments, and waters may influence, even regulate, bioremediation efficacy. The term "bioavailability" means many things in many contexts. This section compiles definitions of bioavailability from nine recent authoritative sources. The quotes that appear below are intentionally extensive to reveal both the many facets of the term "bioavailability" and the biases of the sources.

The first definition (Southgate *et al*., 1989) is taken from a 1988 conference, "Proceedings of Bioavailability 88: Chemical and Biological Aspects of Nutrient Bioavailability," organized by the Food Group of Royal Society of Chemistry, The Working Party on Food Chemistry of the Federation of European Chemical Societies, and the Federation of European Nutritional Societies.

> "Until relatively recently, it has been a common assumption that the nutritional value of foods and diets was more or less synonymous with their nutritional composition as determined by chemical analysis. The limitations of this simple picture first became obvious in relation to trace element nutrition, but recently many lines of research have emphasized the need to understand and quantify the intestinal absorption and subsequent metabolism of all the major nutrient classes...
>
> Bioavailability has been defined as the proportion of the nutrient that is digested, absorbed, and metabolized through normal pathways... This definition has two important corollaries. First (bio) availability is not a property of the diet of food *per se* but the response of the individual to the diet or food. Second, observed (bio)availability,

> therefore, represents an integration of the processes whereby an ingested nutrient becomes available..."

This definition was selected because the authors were scholars concerned with human nutrition—they were not concerned with the scientific and/or political issues of the behavior and fate of environmental pollutants. Yet, the nutritional essence of "bioavailability" establishes themes that will echo throughout this report: **(1) chemical assays of inorganic and organic materials may overestimate what is actually biologically absorbed and metabolized; and (2) bioavailability is not an inherent property of substances under examination, rather bioavailability reflects the response of a biological system to many integrated processes**.

The second definition (Alexander, 1999), from a recent textbook on biodegradation and bioremediation, makes the abrupt transition from human nutrition to pollutant chemicals in soils and sediments. Chemically extractable pollutant compounds are contrasted with biologically available pollutant compounds, as assessed with biodegradation assays. Five theoretical mechanisms are advanced, which suggest that reduced bioavailability of organic soil pollutants for microbial biodegradation is caused by reactions between organic pollutants and the constituents of soils and sediments.

> "The availability of many chemicals is affected by a series of ill-defined, often uncharacterized processes. In some of these processes, the compound is readily evident and can be easily removed from the soil, sediment, or aquifer by conventional extraction procedures. The evidence for reduced bioavailability of these compounds is the marked decline in the rate of biodegradation. In other processes, the compound is still present, but can only be removed from the environmental sample by highly vigorous extraction techniques. The evidence for reduced bioavailability of such a compound is the marked decline in the rate of biodegradation with time or the almost complete resistance of the molecule to microbial destruction...
>
> At this stage in the development of knowledge of a subject that is still characterized by confusion, it might be prudent to envision three separate categories of molecules of reduced bioavailability. These are in addition to compounds that are poorly available because they are sorbed to solid surfaces or present within NAPLs *in the immediate vicinity of microbial cells* having the requisite catabolic enzymes:
>
> (a) Nonsorbed compounds in micropores at some distance from cells having the requisite enzymes...,
>
> (b) Compounds that either enter into, and are retained within, nanopores or that partition into the solids themselves..., and

(c) Chemicals that complex with humic materials or other environmental constituents to form molecular species that, although containing the parent molecules or metabolites generated from them, are in fact new molecular species."

The third definition (Scow and Johnson, 1997), from a review in the soil sciences literature, reiterates that several processes act on organic and metallic pollutants in soils to reduce their toxicological and microbiological availability. An analogy between the availability of native soil organic material and pollutant (xenobiotic) chemicals is drawn.

> "The term bioavailability designates the state of that fraction of a chemical that is available for uptake and/or transformation by living organisms. Although associated primarily with ecotoxicology, and usually in reference to organic and metallic pollutants, the term bioavailability is also relevant to native organic material. Thus, the "problem" of bioavailability, has existed for microorganisms far longer than has the presence of xenobiotic chemicals in the environment.
>
> Sorption, insolubility, and related processes are largely responsible for controlling bioavailability of many pollutants to microorganisms in soils and sediments."

Definition number four (Baveye and Bladon, 1999), derived from a recent international conference on the environmental behavior of organic xenobiotic compounds, evokes the historical, toxicological, and physical-chemical basis for reduced bioavailability. The form of chemicals, the release of chemicals from subsurface environments, and the proportion reaching organisms are emphasized.

> "The concept of biological availability was apparently first proposed in 1975 at a National Science Foundation workshop on ecosystem processes and organic contaminants. and was originally based mainly on physical chemistry… Since then, physical chemists and biologists have developed independent interpretations of the concept. Traditionally, chemists have defined bioavailability in terms of the chemical form in which the compound or element of interest occurs at a given time. Alternatively, definitions derived by biologists have assumed that the chemical form in the bulk phase is relevant only to the presence of a biological receptor; thus, they have defined bioavailability based on the portion of the compound that could pass into an organism under a given set of conditions…
>
> At this juncture, it is not necessary for the sake of our argument to choose between the approach to bioavailability that focuses solely on the amounts of xenobiotics that cross the organism's external boundary…or the approach that relates these absorbed amounts to the capacity of the organism's environment to supply/release the

> xenobiotics… Both perspectives have merits, which have to be assessed on the basis of the measurements that one can perform on organisms in subsurface materials."

Definition number five (Maier, 2000) was prepared for the International Society for Biotechnology. Emphasizing microbial metabolism of organic environmental pollutants, this definition examines the relationships between "perceived" contaminant concentrations and details of microbial metabolism such as nutritional status, enzyme-induction, energy expenditure, and growth. Three causes for reduced bioavailability are mentioned.

> "Bioavailability can be defined as the amount of contaminant present that can be readily taken up by microorganisms and degraded. One reason bioavailability as defined here is so important is that it governs the rates of biodegradation. Bioavailability controls biodegradation because microbial cells must expend energy to induce catabolic gene systems used in biodegradation and if the perceived contaminant concentration is too low, induction will not occur. Indigenous soil microbial populations are typically slow growing organisms often exposed to nutrient poor environments… The presence of contaminants can have a significant impact on the metabolic status of such cells. Three cases can be envisioned that would result from differing bioavailability of contaminants. In the first case, biodegradation will not occur because the amount of bioavailable contaminant is insufficient to justify the energy expenditure to induce biodegradation. In the second case, at low bioavailable concentrations, microbial cells may biodegrade contaminants but in a resting stage rather than a growing stage. While in this case, biodegradation will occur, it will be limited because new cells are not being produced. In the third case, there is enough bioavailable contaminant to induce biodegradation in a growing stage. It is this case that will allow for optimal rates of remediation.
>
> There are several constraints that can limit the bioavailability of organic compounds in the environment. These are low aqueous solubility, sorption, and micropore exclusion."

The sixth definition (Bosma *et al.*, 1997) is from a research article focusing on how mass transfer in soil and aquifers limits the bioavailability of organic compounds. These authors indicate that reduced bioavailability is a likely explanation for inefficient biodegradation of contaminants in "old polluted sites." Three causative mechanisms for reduced bioavail-ability are suggested.

> "Particularly in old polluted sites, part of the contaminants appear to be inaccessible for biodegradation…these observations indicated a reduced availability of pollutants in soils and sediments

> contaminated for a prolonged period of time, pollutant—and not nutrient—availability being the obvious cause. The decrease of the bioavailability in the course of time is often referred to as "aging" or "weathering." It may result from (i) chemical oxidation reactions incorporating them into natural organic matter… (ii) slow diffusion into very small pores and absorption into organic matter, or (iii) the formation of semi-rigid films around non-aqueous-phase liquids (NAPL) with a high resistance toward NAPL-water mass transfer."

Definition number seven (Alexander, 1997) is derived from a text addressing the topic of managing contaminated soils. The author points towards limitations in the use of biodegradation as an assay for the bioavailability of organic compounds in soils because assimilation, toxicity, and biodegradation are distinctive biological processes.

> "A note of caution is required in regard to the term *bioavailability*. It is sometimes considered as synonymous with toxicity to one or another species, sometimes as equivalent to biodegradation by microorganisms, and sometimes as synonymous with uptake or assimilation. A compound may be assimilated and, although toxic, may not cause injury because it is not transported to the tissue, cell or intracellular site where the toxicity can be expressed. A chemical may be taken up into microbial cells but still not be biodegraded because that organism does not contain the requisite catabolic enzymes. Uptake or assimilation is thus a better means of assessing bioavailability, but because of the few studies of uptake per se and the many more of toxicity and biodegradation, the term bioavailability also will be used here to include toxicity and biodegradation."

A recent "state-of-the-science" summary document, addressing the toxicological and environmental impacts of organics, explosives, and metals in soils [the eighth definition (Loehr *et al*., 1997)], stressed that bioavailability is a measure that is dependent on "the receptor, the route of entry, time of exposure, and the matrix containing the chemical." This comprehensive definition attributes diminished bioavailability of contaminants to "physicochemical processes that lead to sequestration." Three categories of bioavailability are suggested. Furthermore, this definition alludes to bioavailability's potential impact on endpoints achieved by remediation efforts.

> "Bioavailability is a measure of the potential of a chemical for entry into ecological or human receptors. It is specific to the receptor, the route of entry, time of exposure, and the matrix containing the chemical.
>
> It is increasingly recognized that the response of an ecosystem or any at-risk population is not controlled by the total concentration of a chemical in the media in which a receptor resides, but instead is

controlled by only that portion which is biologically available. Thus, the definition of "how clean is clean?" or, alternatively, what adverse effects may be exhibited by exposure to chemicals in soils, is largely determined by the physicochemical processes that lead to sequestration and the biological processes that may lead to chemical release and accumulation in an ecosystem. In order to set realistic soil or sediment quality limits for regulatory purposes or to establish endpoints for remediation processes, the physical, chemical and biological mechanisms that govern chemical release and biological uptake from soils and sediments must be more fully understood.

Aspects of bioavailability include:

- *Physicochemical availability*—the fraction of a chemical that is not sequestered and rendered inactive in a solid or other stable phase;
- *Direct bioavailability*—all or part of the non-sequestered chemical that is directly available for entry into specific receptors, depending upon the route and duration of exposure; and
- *Organic-induced bioavailability*—the fraction of a chemical, perhaps initially considered to be sequestered, that is available after processing of the soil/sediment by living organisms.

Ultimately, bioavailability is defined by field conditions and all tests and surrogates for bioavailability must be validated by field measurements."

The final definition (Linz and Nakles, 1997) is taken from a document apparently aimed at using scientific arguments about the behavior of contaminants and their bioavailability in soil to influence environmental regulatory policy. Contaminant release from soil and specific exposure routes for receptors are emphasized. This lengthy excerpt illustrates that it might be possible to extend agricultural, pharmaceutical, and waste stabilization policies toward establishing "environmentally acceptable" concentrations of contaminants in soil.

"The definition of "bioavailability" was also highlighted as important. As defined in this report, the "availability" of contaminants in soils has two components: (1) the rate and extent to which the contaminant is released from the soil into the surrounding groundwater and soil vapor and (2) the rate and extent to which the contaminant is assimilated by ecological and human receptors following dermal contact, ingestion, or inhalation (i.e., bioavailability). The work group stressed the importance of defining "bioavailability" in terms of the specific receptors of interest (e.g., microorganisms, humans, animals) and the likely exposure routes

of importance to these receptors (e.g., ingestion of water or soil or dermal contact with water or soil). This matrix of receptors and exposure routes identifies the biological systems for which the mechanisms of bioavailability should be investigated…

The work group noted that the concept of environmentally acceptable endpoints currently exists within the existing regulatory framework as evidenced by the use of TCLP (Toxicity Characteristic Leaching Procedure) as a means to determine the hazardous classification of wastes. This procedure classifies the potential hazard of a waste based on the fraction of the contaminants that leach from it and not on the total contaminant concentration that is present. Furthermore, the US EPA has accepted waste stabilization and solidification as acceptable technologies for site remediation. This acceptance required the agency to acknowledge that the relative mobility of the contaminants in the waste (without treatment) was reduced following the application of the technologies and that this reduction was permanent over time. These concepts are consistent with this document, namely that hydrocarbons remaining in contaminated soil following bioremediation are not mobile. In effect, the contaminated material has been "biostabilized."

Agriculture and other industries integrate information associated with the presence of chemicals and their toxicological and environmental impact:

- Agriculture. The methodologies for evaluating the delivery and fate of nutrients in soil as well as the fate and effects of pesticides in soil have been formally documented (e.g., pesticides are regulated by the US EPA under FIFRA [Federal Insecticide, Fungicide, and Rodenticide Act]). These methodologies and evaluation techniques may be transferable to the determination of environmentally acceptable concentrations of contaminants in soil.

- Pharmaceutical Industry. The development of pharmaceutical products encompasses many of the issues that must be addressed for contaminants in soil. For example, the development of slow-release capsules required an understanding of the rate of release of the chemicals to the receptor and the response of the receptor to that dose of chemicals. These same issues must be addressed to make the argument that the release of contaminants from soil that are ingested, inhaled, or contacted dermally by humans may be acceptable, even if the concentration of the contaminants in soil is not zero. The methodology that has been followed by the pharmaceutical industry in this area may be applicable to this environmental issue."

3.2. Biosensor Technology Offers a Means Towards Direct Measurement of Bioavailability

Recent biotechnological developments have opened the possibility of directly measuring the bioavailable fraction of chemicals in environmental samples. These developments and their implications are briefly described below.

Many types of microorganisms have evolved networks of enzymes that contribute to metabolic pathways for the transformation of both organic and inorganic toxic contaminant chemicals. Regulation of genes coding for such metabolic pathways is essential for survival and efficient metabolic function of the host microorganism. In a typical genetic regulatory system, gene expression (transcription of mRNA) is activated when the proper inducer compound is sensed in the environment of the microorganism. This type of transcriptional control is achieved through the interaction of a transcriptional activator protein with an inducer compound (often the toxic chemical itself), as well as with RNA polymerase and the DNA that codes for the appropriate detoxifying proteins.

During the past decade, several groups of investigators have used genetic engineering techniques to combine the genes involved in sensing environmental contaminants with reporter genes, such as the *lux* operon, that triggers a light-producing bacterial luciferase reaction. Thus, biosensors of this type can be engineered by placing reporter genes under the control of transcriptional activators (along with their corresponding promoters; Willardson *et al*., 1998). Under appropriate conditions, a direct correlation between the concentration of a contaminant chemical and reporter enzyme activity (e.g., light) can be established. These biosensors have been developed for detection of organic (e.g., Heitzer *et al*., 1994; King *et al*., 1991; Willardson *et al*., 1998) and inorganic (e.g., Corbisier *et al*., 1999; McGrath *et al*., 1999) chemicals in environmental samples.

Because whole bacterial cells are often the detection system in the biosensors, they, by definition, measure the amount of contaminant that is bioavailable. Thus, to some degree these biosensor techniques provide a means of direct detection of bioavailable environmental contaminants. Biosensors offer the potential of being free of the pitfalls characteristic of chemical extraction and analytical procedures.

However, despite possible advantages, biosensors also have limitations. These include: (1) responses specific to the membrane structure and uptake system of the genetically engineered microorganisms (usually *E. coli*). The diverse, uncharacterized microorganisms actually active in soil may differ from *E. coli* in their membrane permeability traits; thus, the biosensor may not be a valid surrogate; (2) the biochemistry and specificity of sensor reactions may not be sufficiently understood to contend with false positive or false negative signals that may occur in complex geochemical settings; and (3) the physical location of biosensor microorganisms is unlikely to realistically mimic that of the native soil microorganisms. Thus, much

progress will likely be required before biosensor measurements will supercede the indirect bioavailability measures discussed above.

3.3 Summary

Bioavailability is a concept that has recently been borrowed from the discipline of nutrition and applied to studies of pollutant compounds. Bioavailability is used, conceptually, to interpret laboratory- and field-based measurements of the behavior of environmental pollutants in the presence of biota (microorganisms and plants) in soils, sediment, or waters. Contrary to many implicit and explicit views (e.g., definition number 8, above, and the promise of biosensors), bioavailability of chemical wastes to soil organisms cannot be directly measured. Instead, bioavailability is an emergent trait, a malleable trait. Information about bioavailability emerges from the context of real-world or laboratory experimental systems containing biota, contaminant compounds, and a geochemical matrix (soil or sediment or water). It is the specific, detailed, three-way interactions between system components that allow inferences about bioavailability to be drawn. Figure 1 provides a graphical conceptual summary of this definition of bioavailability.

Figure 1 displays directly measurable system components. Thermodynamics (large circle) provides a stage for potential reactions that could occur between contaminants (small circle), biota (oval), and soils or sediments (cube). The success of biodegradation processes, hence bioremediation efforts (one-way arrow, down), depends on specific interactions (two-way arrows) between system components. Inferences about bioavailability can be drawn only after system behavior and output (biodegradation/bioremediation) have been observed. Because information about bioavailability is determined by the experimental conditions, the environmental relevance of experimental conditions used in bioavailability assays must be carefully scrutinized.

Based on the nine definitions listed earlier, there are six hypothesized mechanisms that reduce the availability of contaminant compounds for biotic uptake and metabolism in soils, sediments, and waters. These mechanisms, which stem from interactions between the contaminants and the soils or sediments are as follows:

(i) sorption reactions that bind contaminants to solid phase natural surfaces;

(ii) partitioning reactions that place contaminants in non-aqueous phase liquids (NAPLs)—including NAPLs with “semi-rigid” surface films;

(iii) spatial separation of contaminants in micro- or nano-pores;

(iv) complexation reactions that create new covalent bonds between contaminants and humic substances;

(v) insolubility of the contaminants; and

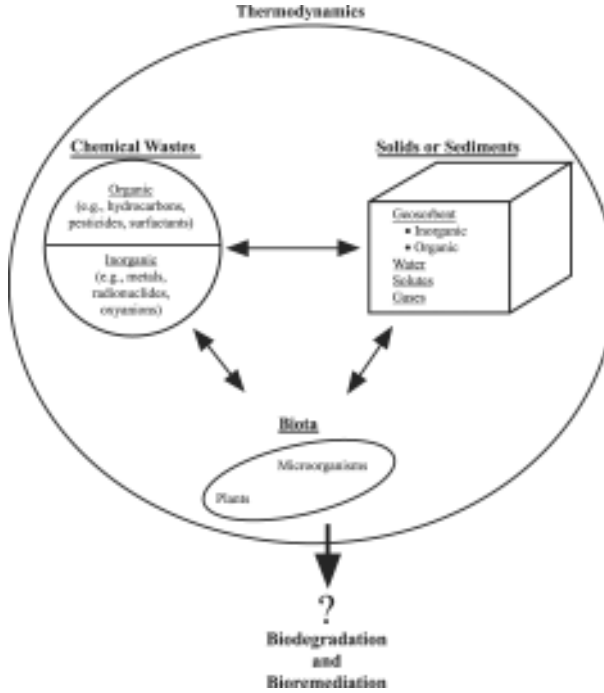

FIGURE 1 Conceptual representation of the relationships between chemical wastes, biota, and soils, or sediments. The bioremediation outcome is governed by thermodynamic instabilities of unique sets of contaminants in unique combinations with biota and soil or sediment. The outcome allows inferences about bioavailability to be drawn (see text, Section 3.3, for explanation).

(vi) partitioning reactions that place contaminants in natural organic matter.

A major goal of this report is to scrutinize these six (and related) hypotheses (see especially, Sections 5, 6, and 8).

Section 4

A Survey of Field Projects Using Biodegradation To Treat Contaminants in Soils

This section is designed to document the efficacy of bioremediation field projects and to pose the question: "Does availability of chemical wastes constrain bioremediation as a site cleanup technology?" A comprehensive summary of the performance of bioremediation in field projects is beyond the scope of the report. A sampling of representative projects is provided in Table 1.[1]

The first five entries in Table 1 describe completed microbial bioremediation projects in which soil was contaminated by mixtures of petroleum-related materials. In some cases, the remediation was implemented on freshly spilled materials (entries 3 and 4). In the other cases, the petroleum was left in contact with soil for many years (precise duration uncertain) before the active cleanup efforts. The cleanup endpoints achieved from these remediation activities (800; 700; <50; 10; 100; and 5,409 ppm, respectively, for entries 1-5 of Table 1) were highly variable. One can make reasonable guesses about causes for the variability. These include: concentrations initially loaded, climatic factors, soil characteristics, degree of mixing, variable microbiological populations, and limited availability of contaminants and nutrients. Distinguishing between these many possible causes is an overarching theme of this report.

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[1]A note on sources. Information in Table 1 was not easy to obtain. Unlike peer-reviewed literature published largely by academicians, the practical results of applied bioremediation technology are largely reserved for reports in the "grey literature," where experimental details and rigorous treatment controls are sometimes omitted. Furthermore, the authors of "grey literature" bioremediation reports may be biased toward favoring the results of their efforts both to please their clients (owners of contaminated sites), and to support claims of the effectiveness of bioremediation technology. Quality of data and experimental design were two of the criteria for selecting entries in Table 1. Another criterion was field realism. Although academic studies may feature high-quality scientific procedures, these may be applied to unrealistic "model systems" whose relevance for the field behavior of contaminant compounds may be suspect.

Entry #6 in Table 1 reports that phenol-contaminated sediment (phenol is a water soluble, readily biodegradable compound) was cleaned up by microorganisms to below 1 ppm; while tars failed to fall below 183 ppm after 71 days.

Studies examining microbial metabolism of pentachlorophenol (PCP)-contaminated soils also appear in Table 1. Entry #7 reported a PCP decline from 416 to 150 ppm after 13 weeks of land farming. In the land-treatment system described in entry #8, PCP declined from 55 to 7 ppm after a nearly 8-year mixture of active and passive treatment.

The next four microbial-related entries in Table 1 (numbers 8-11) describe efforts that attempted to use microbial processes to eliminate polycyclic aromatic hydrocarbons (PAHs) from soils or sediments. One of these studies (Entry #9) revealed that PAHs in coal coking waste were very difficult to analyze: despite mixed aeration in a lagoon, sample variability prevented any trends in biodegradation from being discerned. In the remaining three studies (Entries 8, 10, 11) concentrations of PAHs declined substantially, though residual concentrations were approximately 200 and 159 ppm (for Entries 8 and 11, respectively).

Phytoremediation technology is relatively new to bioremediation. Consequently, documented full-scale field studies are still relatively rare. The majority of reports published to date (e.g., Huang *et al*., 1997; see publications accompanying Cunningham *et al*., 1997; Ensley, 2000; Van der Lelie *et al*., 2001; Vangronsveld and Cunningham, 1998) focus primarily on the ability of plants to accumulate contaminants; whereas case studies on the performance of the technology in significantly reducing contaminant concentrations in field soils are quite difficult to identify. The next three entries in Table 1 (numbers 12-14) are among the few field-scale phytoremediation efforts reported to date. Entry #12 reported that total and leachable lead in a silt loam soil decreased somewhat after treatment with Indian mustard and sunflower plants (478 ppm of Pb remained). Entry #13 provides a record of how a planting of corn and white mustard withdrew some metals from soil (based on tissue analyses) but project performance, in terms of reduction of metals in bulk soil, was not provided. Entry #14 of Table 1 notes that a commercial effort using plants to reduce petroleum hydrocarbon concentrations below a regulatory threshold of 500 ppm was successful.

The final two entries in Table 1 (numbers 15 and 16) are among the many studies devoted to clean up of chlorinated solvent-contaminated groundwaters. The microbiological basis for such bioremediation approaches is either aerobic cometabolism of TCE (to short-lived compounds) or anaerobic reductive dechlorination of PCE, TCE, and daughter products (DCE and VC) to nontoxic ethene (NRC, 2000). Because chlorinated solvents are very volatile (and surface soils are easily excavated) bioremediation technology for chlorinated solvents is usually focused on groundwater (deep) habitats. Entry #15 displays results of a recent engineered bioremediation test over a 12 m distance. Steffen *et al*.'s goal was to prove that injection of an aerobic bacterium could effectively metabolize TCE and related

TABLE 1 Survey of Endpoints Achieved by Field Bioremediation Projects

Entry	Habitat/ Location	Age of Spill	Contaminant	Treatment	Degree of Cleanup	Author's Interpretation	Reference
1	Soil in land farm	PD	Petroleum (diesel fuel, heating oil)	Crushing, sieving, mixing for 1 year in a lined land treatment facility	PAH concentration declined from 10,000 to 800 ppm		cited in (Angehrn *et al.*, 1998)
2	Soil adjacent to petroleum tank farm	PD	Bunker fuel oil	Land farming (plowing and irrigation) for 14 weeks	Total petroleum hydrocarbons declined steadily from 5,000 to 700 ppm	Successful project; 150,000 cubic yards of soil were treated	(Compeau *et al.*, 1991)
3	Soil impacted by New Jersey fuel spill	Fresh	Diesel fuel	Land treatment (plowing and irrigation) for 147 days	TPH concentration dropped from >700 to <.50 ppm	Complete remediation achieved	(Troy *et al.*, 1993)

TABLE 1 Survey of Endpoints Achieved by Field Bioremediation Projects (Continued)

Entry	Habitat/ Location	Age of Spill	Contaminant	Treatment	Degree of Cleanup	Author's Interpretation	Reference
4	Soil surrounding a fuel oil storage tank	Fresh	No. 6 fuel oil	Active treatment: *in situ* mixing, plowing, aeration for 6 months. Subsequent passive treatment for 27 months	TPH concentration declined from 60,000 to 23,700 ppm. TPH concenration declined from 23,700 to 10,100 ppm	Biodegradation during active treatment was more than 6 times more rapid than during passive treatment	(Fogel, 1993)
5	Soil in Southern California	PD	Petroleum hydrocarbons	Land treatment: tilling, plowing	TPH declined from 13,860 to 5,409 ppm in 14 months	Site closure successfully achieved	(Jerger *et al.*, 1993)
6	Sediment in surface impoundment, Plaque mine, LA	PD	Phenol, tars	Lined land farming unit: mixing, aeration for 71 days	Phenol concentration declined from 137 to <1 ppm. Tar concentration declined from 1,455 to 183 ppm	All materials passed screening criteria for closure	(Portier and Christiansen, 1993)

TABLE 1 Survey of Endpoints Achieved by Field Bioremediation Projects (Continued)

Entry	Habitat/ Location	Age of Spill	Contaminant	Treatment	Degree of Cleanup	Author's Interpretation	Reference
7	Soil in Minnesota wood treatment plant	60 years	Pentachloro–phenol (PCP)	Land farming (plowing and irrigation) for 13 weeks	PCP concentration declined from 416 to 150 ppm	Rate declined with concentration; 17,000 cubic yards of soil were treated	(Compeau *et al.*, 1991)
8	Soil in creosote-impacted wood treating site, Northeastern US	PD	PAHs, PCP	Active land treatment: mixing, aeration for 1 year	Total PAH concentration declined from 3,000 to 900 ppm. PCP declined from 55 to 16 ppm. PAHs declined from ~900 to ~200 ppm.	Engineered land treatment was effective in eliminating and stabilizing contaminants	cited in: (Loehr and Webster, 1997)

TABLE 1 Survey of Endpoints Achieved by Field Bioremediation Projects (Continued)

Entry	Habitat/ Location	Age of Spill	Contaminant	Treatment	Degree of Cleanup	Author's Interpretation	Reference
9	Sediment in mixed tank systems	PD	PAHs in coal coking waste lagoon	Mixing, aeration for 5 months	Total PAH concentrations began in the 25 to 50 ppm range and ended at 25 to 500 ppm range	Much analytical variability; conclusions evasive	(Leavitt *et al.*,1991)
10	Soil in Geelong, Vicotria, Australia	PD	PAHs	Land farming (mixing and aeration) for 18 months	Initial PAH concentration was between 0.8 and 174 ppm; average PAH mass declined by >1/3	All PAHs below 2 ppm were persistent and considered not bioavailable. Bioremediation was limited by: low bioavailability, low solubility, low concentration of low MW PAHs, desiccation, elevated pH, and uneven nutrient distribution	(Connolly, 1999)

TABLE 1 Survey of Endpoints Achieved by Field Bioremediation Projects (Continued)

Entry	Habitat/ Location	Age of Spill	Contaminant	Treatment	Degree of Cleanup	Author's Interpretation	Reference
11	Soil and creosote-rich sludge in a wood treating site, Western MT	PD	PAHs	Tillage, nutrient amendment, irrigation for 55 months	PAHs declined from 8,340 to 159 ppm		cited in: (Loehr and Webster, 1997)
12	Poorly drained silt loam soil. Ensign-Bickford Company, Simsbury, CT	PD	Lead (Pb) released during open burn/open detonation activities	Indian mustard and sunflower were planted in a 1.5 acre area	Total average Pb declined from 635 ppm (4/98) to 478 ppm (10/98); leaching assay for Pb showed a decline of 0.95 ppm	Plants phytostabilized the site. Further treatment is planned	Federal Remediation Technology Round Table (frtr) Web Site. Blaylock (1997)
13	Twin Cities Army Ammunition plant, Arden Hills, M N	PD	Heavy metals, antimony, arsenic, beryllium, lead, thallium	Corn and white mustard were planted to 0.2 acre plots. Fertilizers and EDTA were added to the soil	Plant yields and metal tissue content were reported; changes in soil concenterations were not reported	During first year, results were less than anticipated	Federal Remediation Technology Round Table (frtr) Web Site

TABLE 1 Survey of Endpoints Achieved by Field Bioremediation Projects (Continued)

Entry	Habitat/ Location	Age of Spill	Contaminant	Treatment	Degree of Cleanup	Author's Interpretation	Reference
14	Former Chevron Bulk Petroleum processing plant, Astoria, OR	PD	Petroleum hydrocarbons (TPHs)	0.6 acre site was tilled, fertilized, and seeded. Plant species unspecified	Contaminant concentration range in top 2 feet of soil dropped from 170-1,000 ppm to 210-450 ppm	Site closure was obtained after first growing season	US EPA Remediation and Characterization Innovative Technologies (REACHIT) Web Site
15	Shallow sand/clay aquifer in Pennsauken, NJ	PD	VOCs: TCE, dichloroethane, vinyl chloride	305 and 243 liters of a biodegrading aerobic bacterium were injected into groundwater; contaminant loss was measured in both test and control areas	Average concentrations fell from 2.2 ppm to between 0.5 and 0.05 ppm in the test area	Using several evaluation parameters, removal ranged from 44 to 78 %	Steffen *et al.*,1999
16	Deep sand aquifer at Dover Air Force Base, Dover, DE	PD	Tetrachloro-ethene, Trichloroethene	Groundwater samples were analyzed for stable isotope fractionation to document reductive dechlorination of PCE and TCE	DNAPL replenishment of contaminants prevented full quantification of efforts	40 % degradation of TCE in plume compared to source area	Lollar *et al.*, 2001

Abbreviations: PD - age uncertain; probably decades old; TPH - Total Petroleum Hydrocarbons; PCP - pentachlorophenol; PCE - perchloroethene; TCE - trichloroethene; DCE - dichloroethene; VOC - volatile organochlorides; VC - vinyl chloride; DNAPL - dense nonaqueous phase liquid; PAH - Polycyclic Aromatic Hydrocarbon

chloroethenes. The bacterium moved out from the injection well, and average concentrations of volatile organochlorides (VOCs) fell during the 2-day treatment. Residuals ranged from 0.5 to 0.05 ppm. Entry #16 (Table 1) examined a new technique (stable isotope fractionation) as a tool for proving that PCE and TCE were anaerobically biodegraded in an aquifer in which a dense nonaqueous phase liquid (DNAPL) source of PCE and TCE resided. Under the circumstances, complete cleanup could not be assessed—but a significant (40 percent) proportion of the TCE was shown to have been metabolized.

Conclusions about the 16 bioremediation projects shown in Table 1 are: (1) residual contaminants remained in all soils and subsurface waters; (2) mixing and aeration accelerated organic contaminant loss; and (3) in some circumstances, cleanup goals and site closure were achieved.

To augment the survey of field bioremediation projects presented in Table 1, another recent source of information was consulted. In Appendix D of "Treatment Technology for Site Clean-Up: Annual Status Report (Ninth edition)" (EPA, 1999), a summary of treatment technology status-report updates was presented. This site-by-site compilation listed 8 years of additions, changes, and deletions of Records of Decisions for treatment technologies used at US EPA Superfund Sites. Of the 373 changes presented, 62 concerned bioremediation treatment. Of these 62 bioremediation projects, 3 were found to be ineffective in bench-scale biodegradation tests and 9 were abandoned because treatment goals could not be met in the field.

It is clear from the above-mentioned EPA report on site cleanup technologies and from the data in Table 1 as well as from related compilations [e.g., laboratory studies of PAH biodegradation in soils (Hughes *et al*., 1997); field observations of pesticide and other chemical persistence in soil (Alexander, 1997)] that both microbial biodegradation and phytoremediation projects are far from 100 percent efficient. Indeed, even when the soil described in entry 1 of Table 1 was re-spread onto experimental field soils, an average of 82 percent of the oily residues remained 2 1/2 years later (Angehrn *et al*., 1999). The question is "Why?" What causes substances to persist in soil? Certainly bioavailability is one of the candidate explanations for ineffective bioremediation, but the influence of bioavailability must be evaluated simultaneously with other alternative hypotheses. The next section summarizes the past and present understanding of mechanisms by which organic compounds resist microbial metabolism.

Section 5

Mechanisms of Persistence of Organic Compounds

5.1 An Evolutionary Perspective on the Persistence of Organic Compounds

The Oxford English Dictionary defines "persistence" as "the action or fact of persisting; firm or obstinate continuance in a particular course in spite of opposition; continued existence in time or (rarely) in space; endurance; continuous occurrence." Within a biogeochemical context where concern is focused on the behavior of organic compounds in soils, sediments, and waters, "persistent" compounds are ones that, once found, continue to be found. This tendency to persist occurs despite the fact that soils, sediments, and waters in the real world are dynamic, open systems where processes such as dilution, volatilization, photolysis, sorption, advection, and microbial biodegradation all contribute toward the disappearance of organic compounds. Biodegradation is often unique among these processes because enzyme-catalyzed biochemical rearrangements often convert organic compounds completely to nontoxic carbon dioxide (Madsen, 1991). Such microbial mineralization reactions contribute to the growth of microorganisms; thus, such processes are robust.

Millions (Wackett, 1996; Wackett and Hershberg, 2000) of naturally occurring organic compounds (synthesized by plants, animals, and microorganisms) are constantly released into the biosphere. These compounds are continuously destroyed and transported by the above-mentioned processes that prevent persistence. Consequently over broad temporal and seasonal scales, naturally occurring organic compounds are generally maintained in steady-state concentrations in soils, sediments, and waters. A widespread readily biodegradable substance, such as glucose (released by microbial attack of the products of photosynthesis), is turned over rapidly and occurs in soil at very low concentrations; while biochemically resistant soil organic matter recycles relatively slowly and occurs at relatively high concentrations. Soil organic matter is a "persistent" naturally occurring material. A

small subset of naturally occurring organic compounds are toxic (e.g., aflatoxin, botulinum toxin), but occur so rarely or are restricted to such rare habitats (e.g., peanuts, anaerobic foods) that these toxins seldom threaten human health and/or ecosystem function. **In summary, compounds of low (or rare) toxicity in balanced dynamic steady-state are the rule for naturally occurring organic compounds.**

Industrial chemicals are subject to the same forces that govern the concentrations of naturally occurring organic compounds. However, two additional factors need to be considered when attempting to understand the environmental fate and persistence of industrial chemicals. First, some industrial chemicals exhibit newly synthesized, novel molecular structures that are unlike naturally occurring compounds. These may not be susceptible to enzymatic attack and/or may not have provided sufficient selection pressure to allow the assembly of efficient metabolic pathways in microbial systems. In the absence of well-integrated enzymatic systems, microorganisms might not attack novel synthetic chemical structures and persistence could result. Second, industrial chemicals are often released nonuniformly in time and space, often at high concentrations. This patchy, discontinuous distribution can often overwhelm naturally occurring processes that might otherwise successfully maintain a dynamic balance between production and destruction. At high concentrations, even readily biodegradable compounds can noticeably persist in soils, sediments, and waters. Regardless of the tendency to persist, industrial chemicals that are nontoxic (e.g., polyethylene) may not threaten human health or ecosystem function, but the combined traits of persistence and toxicity in an industrial chemical (e.g., PCBs, dioxin) can pose serious environmental dilemmas.

5.2 Placing "Bioavailability" Within the Established Framework of Persistence Mechanisms

In an insightful review written more than a quarter century ago, M. Alexander (1973) prepared a list of factors contributing to the persistence (recalcitrance) of organic compounds. This list was as follows:

I. Property of Organic Molecule

   a. Chemical resists attack by all existing enzymes (proper enzymes have not yet evolved),

   b. Molecule unable to pass through cell wall hence unavailable for enzyme induction and intracellular attack,

   c. Site on molecule for enzymatic attack obscured by intramolecular folding,

   d. Molecule composed of a variety of subunits connected by a variety of linkages (thus it is improbable for all of the proper enzymes to be coincident in time and space),

e. Molecule does not yield energy or carbon for microbial growth (cometabolism may occur, however), and/or

f. Molecule or its products are toxic.

II. Property of environment

a. Environment exceeds the tolerance range of environmental factors affecting survival and activity of organisms ordinarily responsible for mineralization of the compounds. Factors include temperature, pH, moisture, light, salinity, toxins, redox potential, and hydrostatic pressure,

b. Environment lacks growth factors or nutrients essential for microbial activity,

c. Environment inactivates enzymes that would otherwise attack the molecule,

d. Environment is sufficiently voluminous to reduce the concentration of the molecule below a threshold required for enzyme induction and/or microbial activity,

e. Environment renders the molecule inaccessible to enzymatic attack by shielding the molecule in microsites on solids or coating the molecule with inert substances,

f. Environment contains resistant organic substances which form complexes with the molecule, thus rendering the molecule resistant, and/or

g. Environment contains metallic cations which form complexes with the molecule, thus rendering the molecule resistant.

Readers of this report may perhaps be surprised to note that "bioavailability" was not explicitly mentioned in the above listing. Instead, the hypotheses for explaining resistance of organic compounds emphasized properties of the organic molecule and of the environment.

The last four items listed above (II. d, e, f, g under properties of environment) are manifestations of interactions between contaminants and soil or sediment. Moreover, threshold concentration, shielding in microsites, organic complexation, and metallic complexation fully encompass the six bioavailability-based mechanisms identified in Section 3.3.

All six hypothesized mechanisms by which bioavailability controls biodegradation (sorption, NAPL-partitioning, micro- or nanopores, absorption into native organic matter, organic complexation, and insolubility) stem from interactions between the contaminant and the soil or sediment. Therefore, progress in comprehending the influence of bioavailability on bioremediation requires understanding the fundamentals of interactions between contaminants and soils and sedi-

ments (Section 6 of this report) and the mechanisms of biotic uptake of contaminants (Section 7 of this report).

5.3 Illustrating Mechanisms of Persistence: Soil Organic Matter (SOM)

To facilitate the task of interpreting data describing the persistence of chemical wastes in soils (Section 8 of this report), it may be instructive to first review our understanding of the persistence of a ubiquitous, nontoxic naturally occurring material, soil organic matter (SOM). Because of its agronomic significance in maintaining soil physical conditions and nutrient status, decades of scholarly research have been devoted to understand why SOM resists microbial decomposition processes (e.g., Alexander, 1973; Allison 1965; Brady and Weil, 1999; Stevenson, 1994; Stout *et al.*, 1981; vanVeen and Paul, 1981). As will become clear in Section 6, ever-improving models are needed to understand soil systems. A synopsis of the current model for SOM is presented below.

Organic matter in soil is composed of recognizable plant material, unrecognizable plant material in various stages of decay, soil biota (microorganisms, soil fauna), and the persistent material, humus. Humus is divided into two pools (McBride, 1994; Stevenson, 1994): biochemically familiar plant-derived compounds such as polysaccharides, polypeptides, and lignins; and humic substances (amorphous polymeric compounds that are inherently resistant to decomposition). In the three-dimensional soil matrix, the bulk of soil organic matter occurs as water insoluble forms that have been traditionally characterized based on their extractability. Four main "associations" between the inorganic matrix of soil and soil organic matter have been identified (Stevenson, 1994): (1) insoluble macromolecular complexes; (2) macromolecular complexes bound together by di- and tri-valent cations such as Ca^{2+}, Fe^{3+}, and Al^{3+}; (3) organic-clay mineral complexes linked by polyvalent cations (clay-metal-humus), H-bonding, and other ways; and (4) organic substances intercalated within the interlayers of expanding-type clay minerals.

Soil organic matter does not accumulate indefinitely in well-drained soils. Instead, it reaches equilibrium levels governed by several "not entirely satisfactory explanations" (Stevenson, 1994): by the ability of dark, high molecular weight humic substances to resist microbial attack; by nutrient limitations on humic substance synthesis by microorganisms; and by the degree of protection afforded both pools of humus (familiar classes of compounds and humic substances) through associations with polyvalent cations and clays.

The dynamic state of soil organic matter must be recognized. When plant materials reach the soil, the nine main classes of plant-derived molecules are decomposed by microorganisms in the following order of decreasing rates: sugars, starches, simple proteins > crude protein > hemicelluloses > cellulose > fats, waxes > lignins.

Stevenson (1994) has reviewed the considerable information on soil decomposition processes obtained using ^{14}C-labeled plant residues. For soils of the temperate zone, approximately one-third of the plant-derived carbon remains behind in soil after the first growing season, mostly as a mixture of "labile" and "stable" components of humus. Although attempts to measure the age of the stable organic matter fractions in soil have been made (Stout *et al*., 1981; Stevenson, 1994), absolute age determinations are often thwarted by the dynamic nature of humus: the standing pool of humus is continuously decomposed while new humus is re-synthesized. Nonetheless, mean residence times of the stable pool of modern humus have been estimated at several hundred to somewhat over 1,000 years. According to Stevenson (1994), the mean residence time of the "labile" pool of plant carbon (that remaining after the first season of decomposition) is initially short, but then approaches that of the stable, native humus carbon.

The biochemical processes by which high molecular weight humic substances form are not fully understood. Sposito (1989) suggests four stages in the transformation of plant biomass into humus: (1) decomposition of biomass (including lignin) into simple organic compounds; (2) microbial metabolism of the simple compounds; (3) cycling of C, H, N, O between organic matter and microbial biomass; and (4) microbially mediated polymerization of the cycled organic compounds. The principal humus-forming compounds involved in stages (3) and (4) are believed to be phenolic high molecular weight structures, especially lignin constituents, which are converted to a reactive class of compounds containing quinones that readily condense randomly (Flaig, 1975, as cited in Stout *et al*, 1981; Sposito, 1989; Stevenson, 1994). A key trait of humic substances is their dissimilarity to the biomolecules from which they are derived.

Soil management practices have long been known to influence SOM persistence. When either forest or prairie soils are brought under cultivation, a general decline in SOM occurs (Brady and Weil, 1999; van Veen and Paul, 1981). The decline in SOM through cultivation and cropping practices can only partially be attributed to a reduction in the added quantity of plant residues for humus synthesis. It is thought that loss of SOM occurs because cultivation aerates the soil (thus, stimulating microbial respiratory activity) and exposes previously inaccessible organic matter (such as in micropores) to microbial attack (Stevenson, 1994)].

5.3.1 Summary (Soil Organic Matter Persistence)

Key facts related to SOM persistence include:

(i) Plant-derived biomass (consisting of many familiar classes of organic structures) is continually replenished in the soil system;

(ii) Microbial processes convert dead biomass to two humus pools—recognizable (e.g., polysaccharide, polypeptide, and lignin) and nonrecognizable (humic substances);

(iii) Complexation reactions by metallic soil cations influence both pools of humus—thereby rendering them resistant to microbial attack;

(iv) Both pools of humus associate with clay minerals (inert substances)—thereby rendering the humus resistant to microbial attack;

(v) The "humic substances" pool of SOM also resists enzymatic attack because it is composed of a variety of unusual molecular subunits randomly connected by diverse chemical linkages; and

(vi) SOM persistence can be partially overcome by simple mixing of soil—this is thought to boost aeration and accessibility of SOM to microbial attack.

Items (iii)-(v), above, correspond to items II(g), II(e), and I(d) in Alexander's (1973) scheme for explaining persistence (Section 5.2). In addition, entries I(a)-I(d) of Alexander's (1973) scheme contribute, mechanistically, to the persistence of humic substances. Therefore, SOM provides an illustration of the many mechanisms of persistence and their ability to act in concert. Another crucial point about the origin of SOM is that approximately one-third of the initial mixture of naturally occurring biodegradable plant materials entering soil resists biodegradation in the first season of decay. Furthermore, much of this residue enters the stable humus pool with turnover time of hundreds to more than 1,000 years. **To the degree that plant-derived compounds resemble chemical wastes, lessons from the persistence of SOM can guide inquiry into the behavior and bioavailability of chemical wastes in soil.**

Section 6

Paradigms for the Composition and Structure of Soil* and the Physical-Chemical State of Contaminants Therein

Ideally, in all remediation (including bioremediation) scenarios, the reactions of both inorganic and organic contaminants in real-world sites would be known prior to proposing management and technical cleanup procedures. These reactions are governed by: (1) properties of the contaminants (these are of wide-ranging complexity because they may occur as mixtures whose individual components may have multiple reaction pathways); (2) properties of the soils or sediments (geosorbents*; as discussed throughout this section, especially Section 5.4, geosorbents are extremely complex); and (3) interactions between contaminants and geosorbents. In reality, however, site management strategies employ an empirical, iterative, observational approach in which new information is constantly gathered to improve and adjust issues tied to the hazards of contaminants and their removal/detoxification (National Research Council, 2000).

What follows are several discussions designed to foster an awareness of the compounded complexities of contaminant-geosorbent interactions. This awareness will later (Section 8) be brought to bear on the major focus of this report (bioavailability and bioremediation).

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* Note: Information presented in this section will treat the terms "soil," "sediment," and "geosorbent" (Luthy, et al., 1997) as synonyms. It will be assumed that the facts and principles described here apply to all naturally occurring porous geological matrices.

6.1 Soil Complexity

Scholarly inquiry into the intrinsic properties of soil (pedology), separate from their impacts on plant growth (edaphology), developed significantly in Europe, Russia, and the United States in the 19th century (Brady and Weil, 1999). At least two complementary approaches to soil science have progressed simultaneously since then: field approaches to natural history and soil genesis; and laboratory approaches (chemical, biological, mineralogical, and physical determinations) applied to soil samples. Despite advances in both approaches throughout the 20th century, McBride (1994) has written that "much of soil science is empirical rather than theoretical in practice. **This fact is a result of the extreme complexity and heterogeneity of soils, which are impossible to fully describe or quantify by simple chemical or physical models.**"

Soils are natural bodies, whose lateral and vertical boundaries usually occur as gradients between mixtures of materials of atmospheric, geologic, aquatic, and/or biotic origin. Soils are open systems subject to fluxes in energy (e.g., sunlight, wind) and materials (e.g., aqueous precipitation, erosion, deposition, and inputs of organic compounds from activities of plants, human beings, and other animals). Furthermore, soil's intrinsic complexity stems from its nature as an assemblage of solid, liquid, gaseous, organic, inorganic, and biological constituents whose chemical composition and random three-dimensional structure have not been completely characterized. In addition to physical complexity, the microbial (bacteria, fungi, algae, protozoa, and viruses) physiological processes in soil and their multitude of interactions are dauntingly complicated. Compounding the challenge of understanding *in situ* soil processes is the fact that abiotic reactions (e.g., precipitation, dilution, hydrolysis; Section 6.2) also must be considered when attempting to understand soil geochemistry. Furthermore, in a field setting, plants and animals also effect geochemical change.

Attention also must be paid to the fact that soil properties described above are subject to dynamic changes in time and space. No field setting is homogeneous or static. Regarding spatial inhomogeneity, the physical, chemical, nutritional, and ecological conditions for soil biota undoubtedly vary from the scale of micrometers to kilometers. Regarding temporal variability, *in situ* processes that directly and indirectly influence fluxes of material into, out of, and within soil are dynamic. Climate-related influences (such as temperature, sunlight, evaporation, and precipitation) are probably major variables that cause temporal, variations in biogeochemical processes in soil.

6.2 A Thermodynamic Overview of Inorganic Soil Reactions

Lindsay (1979) provided a unifying thermodynamic overview of soil in which dissolved substances in soil solution are in constant dynamic equilibria with six

independent chemical influences: solid mineral phases; exchangeable ions and surface adsorption; nutrient uptake by plants; soil air; organic matter and microorganisms; and water flux. The mineral phases of soil (typically 90 percent of the solid matter) have been described as "rock on its way to the ocean" (Lindsay, 1979). Primary minerals (the parent material from which soils are derived) were often formed under conditions of high pressure and temperature. At the earth's surface, subject to oxidative and hydrolytic weathering, the primary minerals become secondary minerals as ionic species in solution are leached away and the remaining mineral structures seek lower free energy levels in their atomic arrangements. Soils contain numerous minerals, some of which are crystalline, while others are amorphous or meta-stable. These minerals both respond to and control the dynamic pool of dissolved constituents in soil solution. A detailed discussion of soil mineralogy and equilibria is beyond the scope of this report (for this see, Dixon *et al*., 1977; Lindsay, 1979; McBride, 1994; Sposito, 1987**); but it is critical to appreciate that soil and sediment habitats are in constant chemical transition, albeit at rates that are slow in human terms. Many of the mineral components are thermodynamically unstable, and this instability is compounded by additional reaction pathways imposed by (micro)biological processes—especially those driven by plant-derived carbonaceous materials added via photosynthesis (see Sections 5.3 and 6.1).**

6.3 Models of Soil Structural Characteristics and Limitations in Environmental Microbiology

This section will review information describing the three-dimensional arrangements of soil components. Ladd *et al*. (1996) have reviewed relationships between soil components and the biological activity occurring therein. These authors emphasized the vastness in the range of scale of soil constituents (nine orders of magnitude, from atoms to rocks) and the hierarchical features of soil aggregates that form the three-dimensional fabric of soil. Broadly, six size-based categories of aggregation were described (Ladd *et al*., 1996): (1) amorphous minerals develop at the nanometer-to-angstrom scale; (2) clay microstructure colloids form at 10^{-7}m; (3) "quasicrystals," "domains," and "assemblages" form (10^{-7} to 10^{-5} m) between clay, silt, and smaller particles; (4) macroaggregates (0.1 to 250 µm) occur between sand, silt, and smaller particles; (5) macroaggregates (250 µm to 25 mm) occur between gravel, sand, and smaller particles; and (6) clods (> 25 mm) occur between rocks, gravel and smaller particles.

It is the aggregation, the aggregate behavior, of soil that contributes to its complexity. Tisdall and Oades (1982) insightfully presented a schematic model of the aggregate organization of soil (Figure 2). Emphasized in Figure 2 are both the hierarchical scales of soil aggregates and the mechanistically crucial binding agents responsible for aggregate formation. It is clear in Figure 2 that the various components of SOM (Section 5.3, this report) play a major role in creating soil structure. Roots,

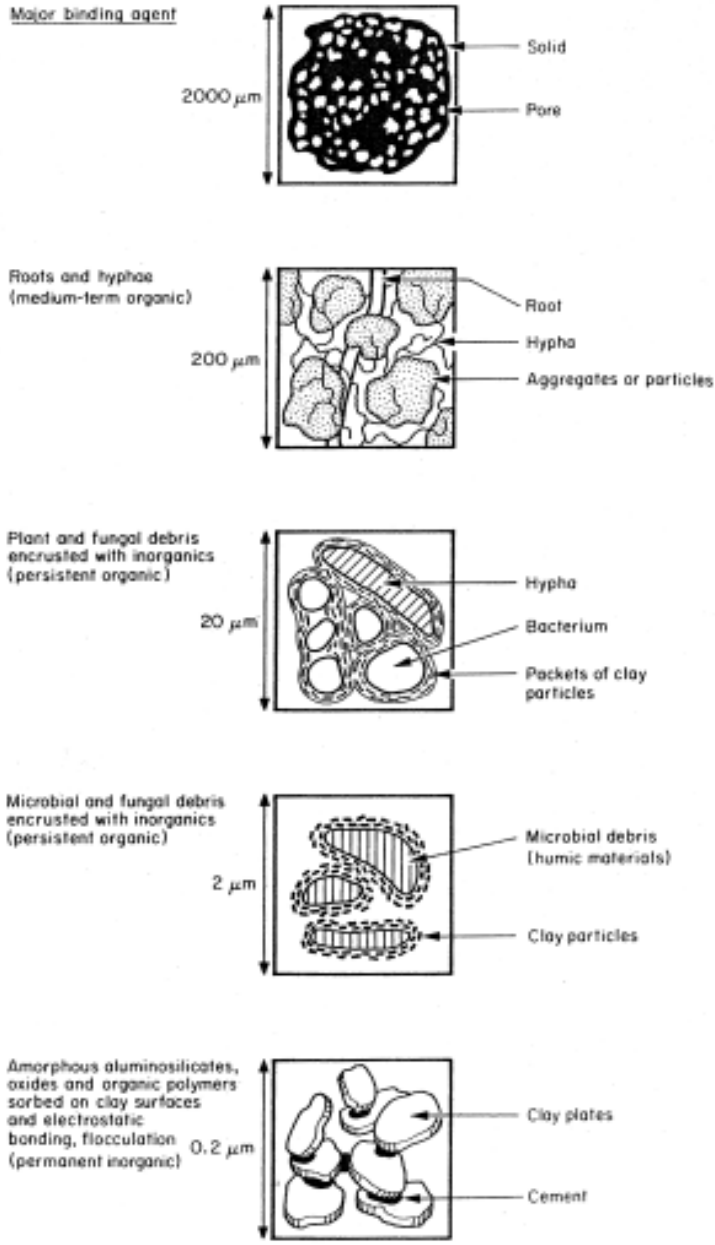

FIGURE 2 Models, at five different scales, of soil components and their contribution to soil structure (from Tisdall & Oades, 1982).

hyphae, plant debris, fungal debris, bacteria, and humic materials are specifically mentioned in Figure 2 because of their structural contributions to the soil matrix.

Documentation of "soil micromorphology" (or "soil fabric"; Ringrose-Voase and Humphreys, 1994) has played a major role in establishing and reinforcing the type of model of soil aggregate organization shown in Figure 2. Microscopic procedures applied, whenever possible, to intact soil samples (Foster, 1993; Ladd *et al*., 1996) include: transmitted light through soil thin sections, transmission electron microscopy (TEM), scanning electron microscopy (SEM), electron microprobe analysis (EMP), and environmental SEM. These approaches have provided direct observations of intimate associations in soil aggregates of solid surfaces, root hairs, fungi, bacteria, extracellular polysaccharides, clay films (cutans), humic substances, and cellular debris. Such "ultrastructural" studies have revealed that the soil biomass occupies only 0.001 percent of the soil volume. Microorganisms, though present in large numbers (approximately10^9 cells per gram) are neither uniformly nor randomly distributed but, as revealed by TEM of soil sections, have been found clumped near or within cellular residues or in micropores (Ladd *et al*., 1996). Electron microscopy/EMP approaches also have been applied to controlled model systems, and provided important insights into the mechanisms by which microorganisms can transform (i.e., precipitate and/or dissolve) inorganic materials (e.g., Warren and Ferris, 1998). However, one well-recognized limitation of high-resolution soil microscopy is that each image surveys such a small soil volume that accruing information truly representative of bulk soil remains a challenge (Foster, 1993).

Within the discipline of environmental microbiology, there has been longstanding interest in understanding small-scale relationships between microorganisms and their habitats. Microscopic environmental microbiological inquiries into the identity and biogeochemical activity of microorganisms have been conducted. The goals of these inquiries have been approached by using various combinations of confocal scanning laser microscopy (for three-dimensional images), autoradiography (to locate both added model radioactive substrates and to document their uptake by microorganisms), fluorescent antibody staining of individual microorganisms, and probing of naturally occurring microorganisms with fluorescent-labeled oligonucleotides that hybridize with 16S rRNA molecules (Amann, *et al*., 1995; Amann and Kuhl, 1998; Ghiorse *et al*., 1996; Lee *et al*., 1999; Ouverney and Fuhrman, 1997, 1999). In a now slightly dated review, Madsen (1996) summarized the accumulating attempts to discover both the *in situ* biogeochemical activities of microorganisms in soil and their identity. Conclusions were that methodological limitations of sample preparation, incubation, and analysis have prevented achieving the goal of knowing "who, what, when, and where" of microorganisms in soil habitats (Madsen, 1996). A recent exception to this statement is the discovery that anaerobic methane oxidation in deep sea sediments can be carried out by an intimate association between sulfate-reducing and methanogenic bacteria (Hinrichs *et al*., 1999; Boetius *et al*., 2000, Orphan *et al*., 2001). Existing methods for growing

and describing microorganisms in soil typically overlook at least 90 percent of those that are detectable microscopically (Amann *et al*., 1995; Madsen, 1998**). Thus, not only has the complexity of the soil habitat prevented its full chemical characterization, but there may be a vast, undiscovered diversity of microorganisms, perhaps with novel metabolic properties, residing in soil. A complete census of naturally occurring microorganisms has never been achieved in any habitat (Madsen, 1998). Moreover, microorganisms responsible for biogeochemically significant processes in natural habitats have almost never been identified (Madsen, 1998).**

6.4 Interactions Between Geosorbents and Contaminants

There is a broad diversity of physical and chemical forces that governs the interactions between geosorbents (soils and sediments) and contaminants. These interactions range from colloidal and electrochemical phenomena to acid-base and sorptive reactions (e.g., McBride, 1994; Sposito, 1989). Given the perhaps "impossible" complexity of soil and sediment systems (Sections 6.1 and 6.3 of this report), it is likely that an accurate and comprehensive understanding of soil reactions will only be approached by combining the results of many types of inquiry. Physical, chemical, mineralogical, and microbiological characterization of extracted soil constituents can augment direct microscopic observations of soil micromorphology (Section 6.3). As was emphasized, conceptually, in Sections 3.1 and 3.3, the central issues of this report focus on the details of specific interactions between specific geosorbents and specific contaminants. Considering the wide-ranging properties of potential geosorbents (e.g., types of organic matter, types of minerals, and their respective abundances) and potential contaminants (e.g., organic, inorganic, soluble, insoluble, ionic, hydrophobic), a thorough examination of the many possible permutations of geosorbent-contaminant interactions is beyond the scope of this report. Nonetheless, both organic and inorganic contaminants will be discussed below. A particular class of organic contaminants, hydrophobic organic compounds, will serve to illustrate how inferences of geosorbent structure can be drawn from indirect observations of contaminant behavior. Examples of direct observations of organic and inorganic contaminants in soils also will be presented.

6.4.1 Inferences of Geosorbent Structure Based on Indirect Observations of Hydrophobic Organic Compounds

In a recent critical review on sequestration of hydrophobic organic compounds (HOCs) by geosorbents (Luthy *et al*, 1997), a group of experts stated that **"currently there are no direct observational data revealing the molecular-scale locations in which nonpolar organic compounds accumulate when associated with natural soils or sediments. Hence, macroscopic observations are used to make**

inferences about sorptive mechanisms and the chemical factors affecting the sequestration of HOCs by geosorbents." Many such macroscopic observations are derived from laboratory operations that: (1) react soil or sediment with a solution of known composition at fixed temperature and pressure for a prescribed period of time; and (2) chemically analyze the reacted soil, the soil solution, or both to determine their compositions (Sposito, 1989). For decades, these types of experiments have been carried out on combinations of HOCs and field-derived or artificial geosorbents. By analyzing the rates and extents of sorption and desorption, while varying key components of the geosorbent [e.g., organic matter, mineral surfaces, and non-aqueous phase liquid (NAPL) content], many investigators have independently developed theories about geosorbent properties. These have often involved mathematical models that explicitly and quantitatively define a variety of "sites" for sorption and desorption reactions based on equilibrium and kinetic interactions between contaminants and geosorbents. These types of analysis have given rise to many theories that contribute explanations for contaminant behavior (e.g., "two-site model," "two site-two region model," "gamma model of continuous distribution of rate constants," "organic matter diffusion model," "sorption-retarded pore diffusion model"; see Brusseau *et al*., 1991; Connaughton *et al*., 1993; Pignatello and Xing, 1996; Scow, 1997; Young and Weber, 1995). These models often evoke physical characteristics of sorbents such as "intraparticle nanopores" (Pignatello and Xing, 1996) and "glassy versus rubbery organic matter" (Graber and Borisover, 1998; Pignatello and Xing, 1999; White and Pignatello, 1999).

The recent consensus critical review mentioned above (Luthy *et al*., 1997) has summarized the "state-of-the-science" for HOC-geosorbent interactions. Figure 3 graphically presents current ideas. Please note that there is a great deal of similarity between Figure 3 (from Luthy *et al*., 1997) and Figure 2 (the soil model presented by Tisdall and Oades, 15 years earlier). The components of Luthy *et al*'s. (1997) geosorbent model (Figure 3) are: mineral-phase geosorbent (the particles); amorphous sorbent organic matter ("SOM" in Figure 3); dense SOM; clay particles and/or oxide coatings; NAPLs (fresh, and aged or weathered); mesopores; micropores; combustion residue (e.g., soot) and water or gas in pores. Each of the model components was carefully scrutinized and justified based on a variety of physical, chemical, and contaminant-behavior techniques.

As summarized by Luthy *et al*. (1997), the key measurement parameters that provide clues to geosorbent properties are: sorption-kinetics (is equilibrium reached rapidly or slowly? is there evidence for hysteresis?); extent of equilibrium partitioning of the contaminant between aqueous and solid phases (is this partitioning linear or nonlinear?); heats of sorption (low or high?); activation energy (low or high?); competition (do other sorbates influence sorbtion reactions?); sorbate characteristics (do molecular steric effects influence sorption?); and extractability (can solvents alter geosorbent behavior by removing key components?). Based on a systematic examination of the patterns of HOC partitioning behavior in the presence of many types of geosorbents,

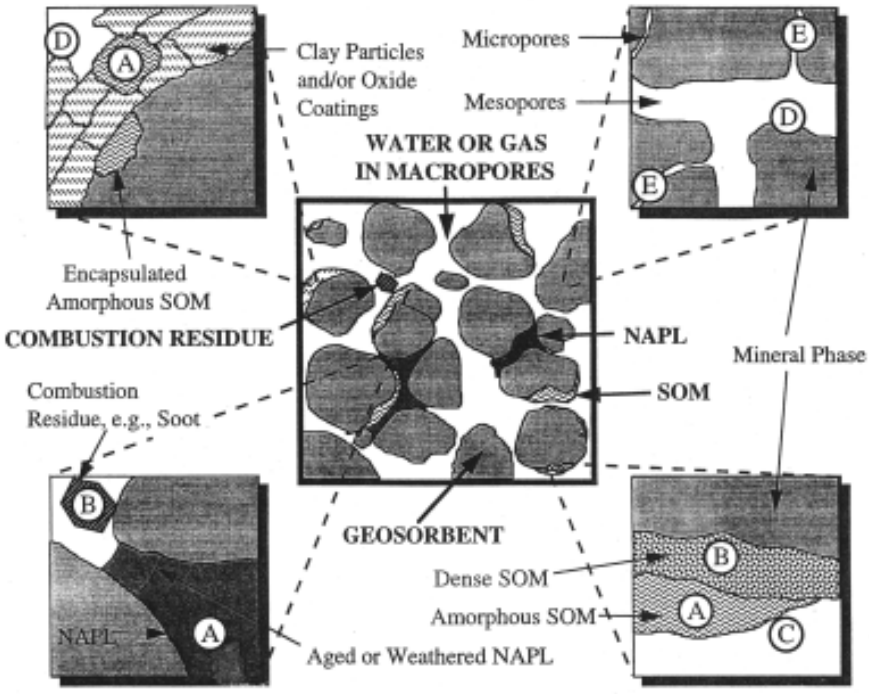

FIGURE 3 Conceptual model of geosorbent domains developed by Luthy *et al.*, (1997). The circled letters refer to representations of sorption mechanisms (described in text, Section 6.4.1.). The geosorbent domains include different forms of sorbent organic matter (SOM), combustion residue particulate carbon such as soot, and anthropogenic carbon including Nonaqueous Phase Liquid (NAPLs).

Luthy *et al.* (1997) presented five hypothetical mechanisms for explaining how HOCs interact with geosorbents. Each of these five mechanisms is represented graphically in Figure 3 as a capital letter from A to E. Case "A" is absorption into amorphous or "soft" natural organic matter or NAPL. Case "B" is absorption into condensed or "hard" organic polymeric matter or combustion residue (e.g., soot). Case "C" is adsorption onto water-wet organic surfaces. Case "D" is adsorption to exposed, water-wet mineral surfactants (e.g., quartz). Case "E" is adsorption into microvoids or microporous minerals (e.g., zeolites) with porous surfaces at water saturation.

The reader should note the correspondence of the five hypothetical sorption mechanisms (letters A to E in Figure 3) mentioned earlier with the six hypothesized mechanisms that reduced bioavailability from Section 3.3. The consensus

critical review by Luthy *et al.* 1997) concluded by recommending that "the discipline is in need of a more sophisticated understanding of…mechanisms at the microscale." Some progress in this area has been recently made (see below).

6.4.2 Direct Observations of Contaminated Geosorbents

Direct, small-scale observations of biological and geological materials have led to profound discoveries in virtually all areas of scientific endeavor (e.g., histology, mineralogy, biochemistry, and the engineering and material sciences) since the invention of the microscope (Goldstein *et al*., 1992). The trend in recent decades has been in at least two directions: toward greater resolution (e.g., atomic force microscopy, angstrom resolution in x-ray crystallography of proteins) and toward combining spectroscopic with visual analyses so that both the spatial arrangement and the composition of materials can be simultaneously integrated.

Although new technical advances in microscopic characterization of environmental materials yield new information, methodological limitations are slow to be fully overcome. As an example, Myneni *et al*. (1999) recently studied the macromolecular structure of humic substances using high-resolution spectromicroscopy at the Advanced Light Source at Lawrence Berkeley Laboratory. These investigators achieved heretofore unattainable image resolution that demonstrated the influence of solution chemistry, mineralogy, and source of origin on the size and shape of humic substances isolated from aquatic and soil environments. Though such details of the macromolecular structure of humic substances were new, the true three-dimensional arrangement of humic substances in intact soil matrices remains virtually unexplored.

6.4.2.1 Hydrophobic Organic Compounds

During preparation of this report, several articles were encountered that directly examined organic contaminants in geosorbent matrices. Examples follow.

Gillette *et al*. (1999) used microprobe two-step laser desorption/laser ionization mass spectrometry (μL^2MS) to identify and characterize trace PAHs on geosorbents. The geosorbents examined included soil spiked with PAHs and also both biotreated and field-derived contaminated samples (soil and sediment). PAH occurrence was mapped across the surface of individual particles at 40 µm increments. Results indicated that sorption phenomena were heterogeneous: individual geosorbent particles seemed to be composed of different subparticle-size regions having different affinities for PAHs.

Ghosh *et al.* (2000) continued the μL^2 MS studies but added Fourier transform infrared (FTIR) micro-spectroscopy and scanning electron microscopy/wave length dispersive x-ray spectroscopy (SEM/WDX) to their inquiry. These multiple assays

were used to examine PAH contaminants across sections of sediment particles derived from Milwaukee Harbor. PAH concentrations in coal- and wood-derived particles were found to be several orders of magnitude higher than on silica particles. Surface analyses revealed that PAHs were associated with organic-rich areas on sand particles, and not associated with bare silica regions. Cross-sectional analyses of particles (silica and coal) showed that PAHs were far more abundant (30 to 100 times) on exterior, compared to interior, regions of these particles. Thus, near-surface sorption processes (<5 μm) and not deeper absorption processes were indicated by this study. Ghosh *et al*. (2000) also fractionated the Milwaukee sediment according to particle size and density. This showed that, although organic material (coal and wood) only comprised 5 percent of the total sediment weight, this fraction contained 62 percent of the PAHs. When PAH desorption from the various sediment fractions was measured, the coal/wood organic material was found to release less than 10 percent of the bound PAHs to Tenax resin. This contrasted with more than 80 percent release of the sorbed PAHs from the silt/clay inorganic fraction. Ghosh *et al*. (2000) suggested that the PAHs associated with wood and coal may not be bioavailable.

In a followup study, Ghosh *et al*. (2001) used desorption kinetics, thermal program desorption-mass spectrometry (TPD-MS), and mathematical modeling to further characterize the behavior of PAHs present in the Milwaukee Harbor sediments. Desorption activation energies and rates were calculated for a range of PAHs. The model that best described desorption was one in which the PAHs were located like a rind on the outer regions of sorbent particles. The authors concluded that PAHs associated with clay/silt particles desorb rapidly and are characterized by low desorption activation energies. In contrast, PAHs associated with coal-derived material desorb at much slower rates and are characterized by high desorption activation energies. The study concluded that "PAHs associated with media having large activation energies may thus comprise the unavailable fraction in sediments and the PAHs may pose less risk than PAHs in clay and silt fraction."

Two other studies will be highlighted here to illustrate recent experimental attempts to explore contaminant-geosorbent interactions. Unlike Gillette *et al.* (1999) and Ghosh *et al*. (2000, 2001) who largely examined geosorbents that were already contaminated, the other studies began with uncontaminated geosorbents and added model PAH compounds.

Guthrie *et al*. (1999) implemented a study designed to determine the structural composition and molecular interactions of pyrene with soluble and insoluble organic matter fractions of sediments. Sediments treated with a biocide and untreated sediments were incubated with [^{13}C]-pyrene in aerated microcosms over a 60-day period. These investigators used pyrolysis-GC/MS and ^{13}C-NMR to follow subsequent interactions with naturally occurring organic fractions (humic acid and humin). The unique spectroscopic probing, ^{13}C-NMR, allowed Guthrie *et al*. (1999) to determine that the added pyrene remained intact throughout the experiment. Yet, during the 60-

day period, the pyrene became increasingly resistant to solvent extraction, while becoming associated with the humic acid and humin sediment fractions. Furthermore, pyrolysis GC/MS indicated that the pyrene-organic matter association involved "adsorbtion or encapsulation (not covalent binding)" and that this association was enhanced in the presence of viable microorganisms (Guthrie *et al*., 1999).

Schultz *et al*. (1999) designed a study to elucidate the relationships between soil organic matter (SOM) structure and sorption behavior of phenanthrene, another PAH. Pyrolysis-GC/MS was used to directly characterize the heat-labile molecular components of SOM in five geosorbents—three surface soils and two subsurface sediments. Principal components analysis of the pyrolyzed fragmentation patterns allowed clear distinctions to be drawn between the types of SOM in each geosorbent. Sorption and desorption parameters were evaluated for the geosorbents, and correlation analyses were performed between the sorptive properties and the soil organic matter components. Phenanthrene thermal desorption profiles also were determined for each geosorbent; these suggested the presence of both mineral and organic-matter components in geosorbents that impede release of PAHs.

6.4.2.2 Inorganic Compounds

Inorganic contaminants [such as heavy metals, metalloids, radionuclides, and oxyanions (e.g., nitrate, chlorite)] exhibit a wide spectrum of properties (e.g., oxidation state, speciation) that allow them to react with inorganic or organic geosorbent components, or both. Many sources of information describing behavior, reactivity, mineralogy, and other aspects of inorganic chemical contaminants have been published (e.g., Adriano, 1992; Alleman and Leeson, 1999; Alloway, 1995; National Research Council, 2000; Vandegrift *et al*., 1992; Vangronsveld and Cunningham, 1998). Efforts to characterize inorganic contaminants in soil have progressed significantly in recent years. Many direct microscopic and analytical procedures have been applied to many combinations of geosorbents and contaminants. Selected examples are presented below.

During uranium processing, aqueous, solid, and airborne radioactive wastes were released to soil at the US Department of Energy's Fernald, Ohio, site. Morris *et al*. (1996) examined contaminated soil samples using a combination of x-ray absorption, optical luminescence, and Raman vibrational spectroscopies, along with ancillary techniques such as energy dispersive scanning electron microscopy and powder x-ray diffraction. The objective was to ascertain the oxidation state, the chemical form, and the physical state (surface precipitate, secondary mineral or absorbate) of the uranium. The procedures used had experimental advantages of: not requiring invasive sample preparation; a spatial resolution range from 1 cm^2 to less than 100 μm^2; and spectroscopic techniques that applied to both amorphous samples and submolecular coatings. The x-ray absorption spectroscopy provided definitive evidence that the bulk of the oxidation state distribution of uranium (75-95 percent)

favored the hexavalent species. Furthermore, the uranium minerals that were identified, similar to autunite and schoepite, often appeared as platey tabular grains ranging in size from 10 to 100 μm or in association with geothite or quartz. Considerable weathering (especially oxidation and both phosphate and hydroxide precipitation) of the initially released uranium had occurred. Additional uranium minerals also were noted, and their photodecomposition properties and field distribution suggested that uranium binding by organic ligands also may have occurred at the Fernald site.

Welter *et al*. (1999) used a combination of XAFS (X-ray Absorption Fine Structure Spectroscopy), XANES (X-ray Absorption Near Edge Structure), and SEM/EDX (Scanning Electron Microscopy/Energy-Dispersive X-ray fluorescence spectroscopy) to examine the chemical speciation of Pb in two soil samples from a battery manufacturing plant in Hanover, Germany. Pb was heterogeneously distributed in the soil, concentrations ranged from between 50 and 140 g/kg. To contend with the inhomogeneity, the soil samples were mixed and ground prior to being analyzed. By comparing XAFS soil signals to those of combinations of authentic Pb minerals, Welter *et al*. (1999) were able to quantify the amount of Pb carbonate, Pb oxide, and Pb sulfate in the two soil samples. SEM revealed individual Pb minerals in the soil matrix.

Galvez-Clouthier and Dubé (1998) implemented a study designed to document the associations between heavy metal contaminants (Pb, Zn, Cu, Cd) and natural sediment constituents in the Lechine Canal, Quebec. X-ray diffraction, TEM (Transmission Electron Microscopy) and other geochemical measures revealed that the sediments consisted mainly of silt- and clay-sized fractions composed of feldspar, kaolinite, chlorite, calcite, and dolomite, as well as minor amounts of Fe minerals, amorphous metal oxides, and organic matter. Each of these constituents bound heavy metals to varying degrees, as assessed by sequential chemical extractions of residual contaminants from oxide-, carbonate-, organic-, and exchangeable-phases of the sediments. The heavy metal partitioning patterns for varying sediment size fractions also were evaluated. Results indicated that no particular mineral phase or size fraction accumulated particular heavy metals. However, significant concentrations of the heavy metals had accumulated in the sediments over the last century, and these posed a high risk for metal release into the water column.

6.5 Summary

Section 6 of this report has presented a glimpse into the facts, principles, and challenges of understanding soil and sediments, and their interactions with organic and inorganic chemical contaminants. Of the multitude of geosorbents, contaminants, and their interactions, only a few were highlighted here. Many of the long-standing questions about real-world geosorbent matrices remain unanswered: they are of complex composition, in complex three-dimensional arrangements, that occur heterogeneously in open biogeochemical systems. Layered onto geosorbent com-

plexity is the biology and biochemistry and ecology of the biota that dwell in soils and sediments. Also, layered onto this are many potential reaction pathways of organic and inorganic chemical wastes. There is simply much more to be learned about geosorbents and contaminants (Sections 6.0 and 6.3), especially because geosorbent composition, contaminant composition, and the geochemical context for biogeochemical reactions are all likely to be site specific.

Despite the above seemingly "impossible" complexities (Section 6.1), both geosorbents and contaminants abide by predictable laws of thermodynamics. We also have robust chemical, mineralogical, and biochemical principles to constrain the potential reactions (Section 6.2). Furthermore, a growing battery of new microscopic and spectroscopic procedures (Sections 6.3, 6.4) are being applied at an accelerating rate to real-world contaminated geosorbents. These procedures have already provided new information that delivers new insights and distinguishes between competing hypothetical models for geosorbents and their dynamic reactions with chemical waste materials. For instance, microscale spectroscopic assays suggest that relatively shallow regions of the "soot" component of sediments feature prominently in governing the release and availability of hydrophobic organic contaminants. Additional progress is likely to be made via an iterative dialogue between hypothesis refinement, application of new analytical technologies, and an accruing database that catalogs the properties of geosorbent matrices and the reactions of contaminants therein.

Section 7

Uptake of Soil Constituents by Plants and Microorganisms

This section will proceed from the general to the specific, from text book principles to recent research articles. After the pivotal role of soil solution in all soil processes is presented, then detailed uptake mechanisms of inorganic and organic compounds by plants and microorganisms will be discussed. Bioavailability issues will be emphasized by focusing on factors that limit accessibility of insoluble materials for transport across the extracellular surfaces of plant roots and microorganisms.

7.1 Principles of Soil Solution Chemistry and Uptake by Plants of Inorganic Compounds

The complexity of the soil habitat was discussed in Section 6.0 of this report (see especially Sections 6.1, 6.2, 6.5). Text in Section 6.2 drew on Lindsay's (1979) observation that **"the soil solution is the focal point. The liquid phase that completely envelops the solid phases…is the medium from which plants absorb their nutrients."**

Regarding inorganic, mineral phase-forming soil components, Lindsay (1979) further stressed that "two very important parameters influence the availability of an element to plants. These are: (1) the intensity factor, which is the concentration of an element in soil solution, and (2) the capacity factor, which is the ability of solid phases in soils to replenish that element as it is depleted from solution." Soils may have a high bulk content of a given nutrient (such as iron) and therefore have high capacity. However, the solution-phase concentration of nutrients is regulated by their dominant mineral form. Often, the most thermodynamically stable mineral phase is the least soluble phase and, if the equilibrium concentration of the stable phase is below the critical concentration for root uptake, plant nutrient deficiency will result. Lindsay (1979) emphasized that nutrient concentrations found in soil solu-

tion are influenced by other key reservoirs, especially solid-phase sorption sites and organic matter. Nonetheless, "mineral phases ultimately control the level of nutrients in solution" (Lindsay, 1979). Thus, characterization of soil minerals and understanding their stability and reactions with inorganic contaminants is crucial for predicting the composition of soil solution. This view was reinforced by Sposito (1989) who stated that "from the perspective of soil chemistry, the bioavailability of an element is determined by competition among plant root systems, the soil solution, and solid-phase particles." Sposito (1989) also emphasized that aqueous chemical complexation reactions influence mineral nutrient bioavailability to plants. Sposito (1989) presented data depicting a direct relationship between the free-ion species of metals in solution and uptake of metals by plants. Sposito (1989) concluded that: "A chemical element is bioavailable, if it is present as, or can be transformed readily to, the free-ion species." Uncharged metal-organic complexes also are generally bioavailable.

As will be discussed below, the above principles of soil chemistry set the stage for understanding nutrient bioavailability in soil. But each component of soil solution has its own set of reaction pathways, solubility products, and spectrum of chemical species. Furthermore, plants and microorganisms sometimes feature unusual physiological adaptations for obtaining specific nutrients from soil solution (especially organic iron-binding agents, the siderophores). Thus, sometimes **elaborate interactions between soil chemistry and soil biota add to the challenge of understanding contaminant bioavailability issues.**

7.2 Movement of Solutes From Soil Solution to Roots

Plant uptake of ionic nutrients requires that they come into contact with the root surface (Havlin *et al*., 1999). There are three primary mechanisms by which nutrient ions in soil could reach the root surface (Barber, 1995; Marschner, 1995): (1) mass flow of ions in solution; (2) diffusion of ions in soil solution; and (3) root interception of solid-phase ions. Havlin *et al*. (1999) present data depicting the relative significance of each of these three mechanisms for the uptake of 12 nutrients by corn plants. Unique soil chemical reactions govern each nutrient; therefore, the relative contribution of each uptake mechanism is highly variable. For instance, Havlin *et al*. (1999) indicate that, for corn, root interception accounts for 1 percent of nitrogen (N) uptake, 3 percent of phosphorus (P) uptake, excessive (171 percent) uptake of calcium, and 11 percent of iron uptake. In contrast, diffusion-based uptake for the same four elements was 0 percent, 94 percent, 0 percent, and 37 percent, respectively.

Mass flow (convection) of nutrients into plant roots occurs when dissolved substances are transported in the flow of water to roots that results from transpirational uptake by the plant. The amount of nutrients reaching roots by mass flow is determined by the rate of water consumption by plants and the concentrations of dissolved soil solution nutrients.

Diffusion-based uptake of nutrients by roots from soil solution is governed by concentration gradients. A high requirement by a plant for a nutrient results in a large concentration gradient, favoring a high rate of ion diffusion from soil solution to the root surface. According to Havlin *et al*. (1999) and Marschner (1995), most P and potassium (K) uptake in corn is governed by diffusion to the root surface over distances of 0.02 cm and 0.2 cm, respectively.

Of the three nutrient uptake mechanisms, root interception is most germane for providing insight into how plants can contend with limited bioavailability. Root interception relies on direct physical contact between plant root surfaces and soil solids. Havlin *et al*. (1999) postulate that root interception occurs via a "contact exchange" mechanism in which overlapping oscillation volumes between ions (e.g., H^+) attached to root hair surfaces exchange with ions held on the surfaces of clay particles and organic matter. Root interception is enhanced both by the growth of new roots and by mycorrhizal infection (caused by a fungal symbiont whose hyphae link root tissue to soil pores) because these processes allow exploitation of greater soil volumes.

Thus far, only movement of soil components to the root surface has been discussed. As a rule, there is great discrepancy between the mineral, nutrient concentrations in soil solution, and the mineral nutrient requirements of plants; therefore, the mechanisms by which plants bring minerals into their tissues must be selective. The selective uptake mechanisms used by plants can include (Marschner, 1995): unique cation-binding properties of rhizodermal cell walls; and ion channels, transmembrane pumps, or protein carriers residing in tonoplasts and/or plasma membranes within plant cells. Marschner (1995) has discussed plant physiological and anatomical mechanisms that confer such selectivity. Details are beyond the scope of this report. Nonetheless, it should be recognized that results of solute-uptake studies performed on both lower and higher plants demonstrate the following characteristics (Marschner, 1995): (1) selectivity—preferential uptake and/or exclusion; (2) accumulation—concentrations of solutes can be much higher in the cell sap than in soil solution; and (3) genetic variability—distinct differences in solute-uptake traits among different plant species.

7.3 Examples of Nutrient Uptake by Plant Roots

This subsection of the report directs readers towards two examples of the detailed mechanisms by which plant physiology and soil-solution chemistry interact to govern solute uptake by plant roots. The first example, phosphorus, has a fixed oxidation state (+5) but occurs in anionic forms in soil solution that form insoluble minerals (e.g., hydroxy-apatite). These anionic forms can bind and/or coprecipitate toxic metals such as Ni, Pb, and U. The second example, Fe, undergoes redox reactions, and has several competing biotic and abiotic reaction pathways in the soil habitat.

7.3.1. Phosphorus (P)

According to Barber (1995), the phosphorus content of soils can vary from 0.02 to 0.5 percent, with an average of approximately 0.05 percent. The phosphorus pool is divided into four general categories: (1) P as ions and compounds in soil solution; (2) P adsorbed onto surfaces of inorganic constituents; (3) P minerals, both crystalline and amorphous; and (4) P as a component of soil organic matter. In soil solution, the dominant chemical species are usually either $H_2PO_4^-$ or HPO_4^{2-}, depending upon ambient pH. This solution-phase P equilibrates rapidly (labile P) and slowly (nonlabile P) with the adsorbed P pool. Barber (1995) indicates that controversy exists over the amount of P that may be adsorbed on the surfaces of such soil constituents as iron and aluminum oxides versus the amount that is precipitated as discrete mineral forms. Many potential P minerals may exist in soil. In basic soils (above pH 7), calcium phosphates (e.g., fluoro-apatite, hydroxy-apatite) should be dominant, while in acid soils (pH<7) iron (e.g., strengite) aluminum phosphates (e.g., variscite) are the dominant forms. Solubilities of the pure crystalline minerals, and their pH dependence can be used to predict the behavior of P in soil. However, kinetic barriers to reaching equilibrium in soil, formation in impure minerals, and metastable states limit the usefulness of such predictions (Barber, 1995).

One-half or more of the total P in many surface soils may be in the organic form—principally as esters of orthophosphate (e.g., inositol phosphates, phospholipids, nucleic acids) (Havlin *et al*., 1999; Barber, 19995). The release of organic P into soil solution (where it can react with other soil constituents or move to the root and be absorbed) is controlled by the rate of soil organic matter decomposition. Barber (1995) states that, in temperate climates, an organic matter mineralization rate of approximately 2 percent per year is to be expected.

7.3.2. Iron (Fe)

In contrast to phosphorus, iron is cationic and undergoes oxidation/reduction reactions. In soil, iron can exist in the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. This variability in oxidation state, combined with a wide range of complexation and precipitation reactions (all dependent on pH, redox, and concentrations of soil anions such as hydroxide and carbonate), contributes to the intricacies of iron's behavior in soil (McBride, 1994; Sposito, 1989). In fact, there are more than nine different possible iron soil minerals (Schwertmann and Taylor, 1977). Iron minerals commonly found in soils are the oxides or hydroxides (goethite, hematite, lepidocrocite, maghematite, magnetite, and amorphous forms). According to Barber (1995), the latter is possibly the most significant form in supplying iron for uptake by the plant. Although Fe^{2+} is more soluble than Fe^{3+}, both oxidation states have such a strong tendency to form insoluble minerals so that total Fe in soil solution is insufficient to meet plant nutritional requirements. Clearly mechanisms besides

simple uptake of iron from soil solution exist—otherwise, plants grown on almost all soils would be Fe deficient (Havlin *et al*., 1999).

There are three primary mechanisms by which plant roots actively increase the availability of iron in soil (Hartwig and Loepert, 1993): (1) exudation of protons to locally solubilize Fe at low pH, (2) release of reducing agents that convert highly insoluble Fe^{3+} to more soluble Fe^{2+}, and (3) by exudation of siderophores (organic Fe-chelating agents that bind and mobilize Fe^{3+}). Plants have been classified as either Fe efficient or Fe inefficient. The Fe-efficient plants respond to iron deficiency by releasing protons and reductants into the rhizosphere where the root hairs absorb the iron, primarily as Fe^{2+} (Barber, 1995). After transport to the protoxylem and metaxylem, the Fe^{2+} can be oxidized back to Fe^{3+}, chelated with citrate and moved into the xylem for transport to the plant shoot (Barber, 1995). According to Tagaki (1993), the scheme just described is "Strategy I" for overcoming iron deficiency; it can involve enzymatic reduction of Fe^{3+} to Fe^{2+} and is prevalent in angiosperms (seed producing plants).

The third mechanism for enhancing iron uptake involves production of siderophores in the roots of grasses. In Tagaki's (1993) scheme, these are "Strategy II"-type plants. The siderophores are organic chelating agents with such high iron-binding affinities that they solubilize the Fe^{3+} from soil minerals. Once in solution, movement of the iron-chelate complex is via mass flow or diffusion back to the root tip region (Barber, 1995), where the Fe is released from the siderophore by being reduced to the ferrous form, and absorbed by the plant root. Transporter-mediated internalization of Fe-siderophore complexes (Roemheld and Marschner, 1986) also has been reported. After being freed following uptake by the root, the siderophore is released to the soil solution where it can again solubilize ferric iron from the mineral phase.

7.4 Contrasts Between Substrate Uptake Mechanisms in Microorganisms and Plants

Soil is inhabited by many types of microorganisms [bacteria, fungi, algae, protists (e.g., protozoa) and viruses]. Most of the discussion below will be restricted to bacteria because of their relevance to bioremediation. There are many major structural, evolutionary, and physiological distinctions between plants and microorganisms (e.g., Madigan *et al*., 2000; Lengeler *et al*., 1999). Only a few of these distinctions are relevant to the uptake of substrates in the soil habitats. Implicit in the previous discussion of root-uptake mechanisms was the fact that the materials brought into the plant tissue from soil are used in assimilative metabolism, e.g., incorporated into plant structural elements (e.g., cell walls, enzymes, and other cytoplasmic components). Microorganisms have assimilative nutritional needs that are similar to plants. However, unlike plants, the majority of soil microorganisms are nonphotosynthetic—their physiologies rely on uptake and metabolism of organic

carbon, not CO_2. This means that soil microorganisms must bring both inorganic and organic substances across cell walls and cell membranes to grow.

Microorganisms live in intimate contact with soil solids. This direct contact with soil solids (analogous to the "root interception" uptake mechanisms for plants) is an important means by which bacteria and fungi acquire insoluble organic and inorganic substances from the extracellular milieu. In addition, microorganisms (especially bacteria) also have dissimilatory nutritional needs—the primary example of this is in the generation of ATP using many different final electron acceptors. Like plants, aerobic microorganisms use oxygen, a sparingly water-soluble gas that readily diffuses across cell membranes, in respiratory processes to generate ATP. Because solution-phase oxygen readily passes into cells, one would not expect enhanced-transport systems for oxygen to be present in the microorganisms. Specific adaptations aimed at overcoming solid-phase bioavailability limitations of the highly soluble alternative electron acceptors (e.g., nitrate and sulfate, used by anaerobic microorganisms) also would not be expected. However, in anaerobic settings, alternative bacterial final electron acceptors can include the highly insoluble oxides of iron and manganese. The mechanisms by which bacteria metabolize these latter insoluble compounds are particularly germane to bioavailability issues.

Because of the importance of solid phase biogeochemical processes, the mechanisms of electron transfer by microorganisms to poorly soluble minerals for anaerobic respiration is subject to intense, ongoing study (Lovley 2000; Newman and Kolter, 2000; Seeliger *et al*., 1998). Currently accepted potential mechanisms (Lovley, 2000) include extracellular electron shuttles either involving naturally occurring humic substances (Lovley *et al*., 1996) or bacterially excreted quinones (Newman and Kolter, 2000) and direct enzymatic reduction of solid oxides at the cell surface via membrane-bound enzyme systems that span the inner and outer cell surfaces.

Thus, it is important to recognize that in bacteria (prokaryotes), the electron transport chain used in ATP generation is located in the plasma membrane at the periphery of the cell (Ehrlich, 1995; Lengler *et al*., 1999). This contrasts with eukaryotic cells (such as plants) in which electron transport occurs internally in special organelles called mitochondria. Because of their specialized cellular architecture, bacteria endowed with appropriate oxido-reductases (enzymes that transfer hydrogen atoms or electrons) in their cell envelope are able to oxidize or reduce insoluble substrates that cannot be taken into these cells (Ehrlich, 1995; Lengler *et al*., 1999). Such substrates include elemental sulfur, iron sulfide, iron oxides, and manganese oxides. **Because the essential enzymes that recognize and act on insoluble substrates are located in exterior regions (e.g., the periplasmic space, the plasma membrane, or the outer membrane), bacteria are uniquely capable of metabolic activities that render extracellular materials "bioavailable." Utilization of endogenous (quinones) and exogenous (humic) substances also can facilitate physiological reactions between bacteria and "nonbioavailable" substrates.**

7.5. Membrane Transport in Microorganisms

[The following discussion is derived largely from Lengeler *et al*., 1999.] Cell membranes define the internal metabolic system of living cells. Membranes act as a physical barrier between microorganisms and their environment. Membranes also carry out essential metabolic, sensory, and reproductive functions. Lengeler *et al*. (1999) state that: "The cytoplasmic membrane of bacterial cells consists of a phospholipid bilayer, which functions as a permeability barrier for most solutes. Polar solutes (e.g., carbohydrates) and charged molecules (ions, carboxylic acids, amino acids) have a very low rate of passive flux across lipid bilayer membranes. In addition to some small solutes and molecules (such as water, ethanol, ammonia, or oxygen), only apolar (hydrophobic) compounds (e.g., phenylamine, glycerol, or fatty acids) pass across lipid bilayers. Bacteria, however, need to transport solutes at high rates across the cell wall and cytoplasmic membrane for growth and metabolism. The solute transfer across bacterial membranes is mediated by specific membrane proteins called transporters, transport systems, carriers, or, in analogy to enzymes, permeases. By means of these proteins, the transfer rate across bacterial membranes can be significantly increased... The presence and activity of carrier systems in a relatively impermeable membrane is the reason for observed concentration gradients of solutes across the cell membrane. These range from 10-30 fold (external/internal Na^+ and internal/external K^+) to more than 10,000 fold (external/internal free Ca^{2+}), up to a 200,000-fold accumulation of some solutes in the cytoplasm (e.g., maltose and particular amino acids)."

Lengeler *et al*. (1999) have further summarized the four key mechanisms of solute transport into prokaryotic cells: (1) diffusion (with or without facilitation by a permease); (2) secondary transport, in which a permease combines with an electrochemical gradient to adjust concentrations across the membrane; (3) primary transport, in which solute transport is directly coupled to chemical or photochemical reactions; and (4) group translocation, in which the solute consumes ATP through phosphorylation reactions inside the membrane. Diffusion-based transport applies to gases (O_2, CO_2, NH_3), small molecules (water, ethanol), and also hydrophobic (lipid soluble) molecules such as aliphatic (e.g., butanol) or aromatic molecules (e.g., benzene). A number of solutes are membrane permeable in their uncharged form (e.g., protonated organic acids) but do not pass through membranes if charged.

7.6. Uptake of Insoluble Organic Substrates by Microorganisms: Wood

High molecular weight organic polymers such as cellulose and lignin are insoluble. These would appear to be "unavailable" for metabolism, yet they are widespread in nature and are biodegradable. Lengeler *et al*. (1999) have described some

general physiological adaptations that allow microorganisms to exploit the diversity of naturally occurring polymeric organic substrates. The microbial world features a wide variety of usually hydrolytic extracellular enzymes that cope with crystallinity, low solubility, and the association of polymers with solid-phase materials.

Cellulose is the most common organic substrate in nature. It is a linear polymer of 100 to 100,000 glucose units linked by ß-1,4 bonds. Cellulose chains form intramolecular and intermolecular hydrogen bonds that allow rigid insoluble fibrils to form. When attacked, this insoluble substrate is eventually converted to soluble glucose monomers and dimers (cellobiose) that, after cell entry, are intracellularly metabolized. Attack of cellulose occurs through the action of a battery of extracellular exoenzymes. These enzymes fall into two general categories: endoglucanases (that sever the linear cellulose polymer, presumably by random attack at sites within the chains) and exoglucanases that sequentially remove dimeric cellobiose units from one end of the chain) (Bayer and Lamed, 1992). According to Lengeler *et al*. (1999), the binding of exoenzymes to surfaces of the microbial cells, the attachment of the enzyme to the cellulose, and possibly the uptake of polymeric substrate into the cell periplasm (between cell wall and cytoplasmic membrane) minimizes both dilution of the enzymes and losses of hydrolyzed cellulose subunits to competing microorganisms.

The anaerobic bacterium, *Clostridium thermocellum*, has evolved a highly specialized multienzyme cellulase complex, termed a cellulosome. This extremely high molecular weight structure (>2 million daltons) is positioned at the interface between the microorganism and the cellulosic substrate. Strong adhesion between *C. thermocellum* and the cellulases is required prior to cellulose degradation (Bayer and Lamed, 1992).

Natural structures like wood are highly complex—consisting of many types of polymers and monomers in a rigid matrix often bound by lignin. Lignin molecules are not susceptible to hydrolytic cleavage (Lengeler *et al*., 1999). Rather, lignin metabolism (rare in bacteria, more common in fungi) requires an oxidizable substrate (e.g., glucose) and O_2 to form hydrogen peroxide that activates ligninase enzymes. Thus, a mingling of different microorganisms, each with their own unique properties, contributes to successful biodegradation of naturally occurring, wood-derived materials.

7.7. Phosphorus and Iron Uptake by Bacteria

Phosphorus is a growth-limiting element under many environmental conditions—soil solution concentrations are in the micromolar range, while intracellular concentrations are approximately 1,000-fold higher (Lengeler *et al*., 1999). Inorganic P (orthophosphate) is the preferred P source in bacteria, as its presence suppresses expression of genes involved in the acquisition of alternative (organic) P sources. Inorganic P can enter the bacterial cell either through low-affinity (per-

mease-based diffusion) or high-affinity (ATP-dependent) transport systems. The latter are activated by inorganic P limitation.

Microorganisms (especially bacteria and fungi) use mechanisms much like plants to meet their metabolic demand for insoluble iron in soil. According to Hofte (1993), with only a few exceptions, all aerobic and facultative anaerobic microorganisms that have been critically examined produce iron siderophores. These fall into three categories based on molecular structure: catechols, hydroxamates, and carboxylic acids. The overall physiological function of microbial siderophores resembles that described for plants (Section 7.3.2); however, some details are distinctive. In an example described by Page (1993), ferric iron was scavenged from soil; the siderophore-bound iron was recognized by the cell that produced the siderophore through specific receptor proteins located in the cell membrane; the ferri-siderophore was transported into the cell and deferrated by mechanisms that involved iron reduction or ligand hydrolysis.

7.8. Summary

Aqueous-phase soil solution represents a conduit for dynamic equilibrium and transport processes that are governed by a combination of organic, inorganic, biochemical, and mineralogical reactions. The soil physical and chemical environment establishes the context for metabolism, growth, reproduction, and nutrient uptake by plants and microorganisms. Plants and microorganisms dwell in this soil habitat whose chemical composition is vastly different from their own.

To acquire key inorganic nutrients, plant roots combine three general uptake mechanisms (mass flow in solution, diffusion, and solid-phase interception) with sometimes highly selective membrane transport carriers (active and passive) to regulate the intracellular composition of cellular constituents. Microorganisms also feature unique structural and physiological traits that allow them to contend with the soil environment. Microorganisms differ from plants in several major ways. Most soil microorganisms have heterotrophic nutrition—thus, they must acquire both organic and inorganic materials from soil. Also, in prokaryotic cell architecture, membrane-associated electron transport systems reside near the cell periphery. This allows some prokaryotes to use insoluble soil components such as Fe and Mn oxides as physiological final electron acceptors. Endogenous (quinones) and exogenous (humic) substances also can facilitate extracellular electron transport reactions for bacteria. Another key characteristic of bacteria and fungi is their ability to produce extracellular enzyme systems that allow insoluble substrates, such as cellulose and lignin, to be degraded to constituent components prior to their uptake and intracellular metabolism. One remarkable ability, shared by microorganisms and plants, is an adaptation that allows them to contend with the unavailability of the essential nutrient, Fe. Both plants and microorganisms acquire otherwise insoluble Fe^{3+} by producing highly specialized, organic chelating agents (siderophores) that solubilize mineral Fe^{3+} and mobilize it to the cell surface. At the

cell surface, the Fe-siderophore complex is destabilized so that, as the Fe is utilized intracellularly, the siderophore cycles back in soil solution to bind more Fe.

Key insights into the bioavailability of chemical wastes in soil may be achieved from fundamental knowledge of the mechanisms by which microorganisms and plants bring soil constituents into their cytoplasm. Highly specialized physiological mechanisms for cellular uptake of some essential soil nutrients have evolved in both plants and microorganisms. Because much microbial metabolic diversity remains undiscovered (see Section 6.3), the full spectrum of microbial uptake systems in soil has almost certainly not been described. To the degree that chemical wastes behave as essential nutrients, one would predict that efficient contaminant uptake could be achieved. Conversely, to the degree that the properties of chemical wastes differ from essential nutrients, one would predict low-efficiency uptake by plants and microorganisms.

Apolar hydrophobic organic compounds in soil should readily pass through cell membranes; hence, they face no biological transport barriers for cellular uptake. In contrast, efficient uptake of polar organic and inorganic contaminant compounds can have a low rate of passive flux into cells. Mechanisms that enhance the uptake (e.g., permease or ATP-driven) of polar contaminant compounds could theoretically increase rates of transport into plants and microorganisms, but enhanced uptake would not be expected unless the contaminants were fortuitously recognized by the uptake systems.

Plants and microorganisms have developed mechanisms for uptake of materials from solid phases in soils; thus, entry into soil solution (by dissolution, desorption, or exchange from soil solids) is not required for acquisition, accumulation, and/or metabolism of chemical wastes by plants and microorganisms. For those who want to use bioremediation processes for soil cleanup, solid-phase substrate uptake capabilities may provide grounds for optimism. However, a less optimistic argument also can be made. The soil habitat poses many constraints on the uptake of soil constituents by plants and microorganisms. The evolution of elaborate solid-phase uptake mechanisms by soil inhabitants can be taken as long-term evolutionary evidence for bioavailability limitations in soil. The subject of bioavailability limitations and their alleviation will be presented in Section 9 of this report.

Section 8

Reviewing the Facts: Examining Relationships Between Contaminant Sequestration and Bioremediation

A recent computerized search of the peer-reviewed literature, using "bioavailability" and "bioremediation" as key words, retrieved hundreds of references that were published over the last two decades. If the goal of this report, "a concise compilation of current knowledge," is to be achieved, clearly much of this literature must be passed over, while focusing on influential research of the highest quality. But, as will become evident in Section 8.1, even a select subset of the literature on relationships between biodegradation of organic compounds and bioavailability provides a confusing mass of seemingly conflicting data and interpretations. This section aims to sift through, evaluate, and weigh the significance of key reports. Background information accrued in Sections 2-7 of this report and arguments developed in Section 8.2 will serve as evaluation criteria.

8.1 Ambiguity Is the Rule: An Historical Overview of the Impact of Solid Surfaces on Microbial Activity

There is a long, substantive history of investigations that have examined how microorganisms respond to the presence of solids—both as particles suspended in an aqueous milieu and as an unsaturated porous geochemical matrix. Much of this early scholarly information was summarized by Marshall (1976). This excerpt from his book appeared under the heading "Effect of soil particles on microbial metabolism" within the chapter entitled "Nonspecific interfacial interactions in microbial ecology: terrestrial ecosystems,"

> **"Adsorption of organic substrates to soils depends on the nature of the particulate matter, the organization of the fabric, the clay types, and the cation status of the soils, as well as on the concentration and**

> **molecular structure of the substrate. The availability** (emphasis by Madsen) **of substrates to soil microorganisms may be enhanced or reduced by the presence of soil particulate matter. This may depend on buffering effects…or on direct sorption phenomena. If the substrates are sorbed, then availability will depend on substrate location relative to that of the appropriate decomposer microorganisms, on whether extracellular enzymes are involved and whether these are sorbed, and on the configuration and juxtaposition of the substrates and enzymes in the sorbed state. Some contradictory results have been reported on the metabolism of specific organic substrates in different soils, but these probably reflect the complexity of soils as microbial ecosystems." (Marshall, 1976)**

Marshall's (1976) comment about "some contradictory results" will appear as a euphemism when compared to summary statements published 14 years later by van Loosdrecht *et al*. (1990). In this influential review entitled "Influences of interfaces on microbial activity," van Loosdrecht *et al*. (1990) state: "Although there appears to be a qualitative consensus that surfaces do influence bacterial metabolism, the experimental observations are not always consistent; neither has a general explanation been advanced for this influence. The lack of experimental consistency is at least partly due to the great variation in experimental design with respect to the nature of the solid phase, the bacteria, the substrate, sterility, and other experimental conditions. The relevant but disparate literature is summarized in Table 1, wherein it is confirmed that, although generally surfaces do exert an influence, no systematic trends can be observed."

Table 1 of van Loosdrecht *et al*. (1990) was entitled, "Summary of the literature on the influence of solid surfaces on microbial behavior." Within this table, the following categories of observations appeared: "increased growth rate," "decreased growth rate," "increased assimilation and decreased respiration rates," "increased respiration," "decreased respiration," "increased adhesion of active cells," "higher activity of attached cells," "decreased substrate utilization," "lower substrate affinity," "change in pH optimum," "difference in fermentation pattern," "increase in productivity," "decreased mortality," and "no effect."

van Loosdrecht *et al*. (1990) offered the following concluding remarks: "The presence of surfaces may positively or negatively (or not at all) affect microbial substrate utilization rates and growth yields. The results often depend on the nature of the organism, the kind and concentration of substrate, and the nature of the solid surface. In interpreting the effect of surfaces on bioconversion processes, all possible physical and chemical interactions (e.g., diffusion, ad- and desorption, ion-exchange reactions, conformation changes, etc.) of a given compound and its possible metabolites with a given surface have to be considered before general conclusions can be drawn."

Particularly germane to this report, Mihelcic *et al*. (1993) published a review entitled "Bioavailability of sorbed- and separate-phase chemicals." This compilation

was motivated by the view that "Hydrophobic organic compounds may be sorbed to soils and sediments or present in a separate phase (e.g., oil and coal tar). Consequently, the effectiveness of bioremediation of soil contaminated with organics may be affected by physicochemical processes that control phase partitioning between solid and liquid, and subsequent solute accessibility to microorganisms. Thus, the bioremediation of soils contaminated with hydrophobic solutes, may depend on the rate and extent of desorption from a solid surface or dissolution from a separate phase." Mihelcic *et al.*'s (1993) assessment of the literature began with the above premise and thoroughly compiled information addressing: field and laboratory observations, associations of microorganisms with surfaces, microbial utilization of sorbed and separate-phase substrate, effects of surfactants on biodegradation, and models that combine sorption and biodegradation. Throughout their review, Mihelcic *et al.* (1993) emphasized the complexity of soil-based experimental systems, potential experimental artifacts, the range of microbial adaptations to biodegradation reactions, equivocal experimental results, and the need to carefully evaluate data.

The first paragraph of Mihelcic *et al.*'s (1993) summary section reads as follows: "The investigation of the physical, chemical, and biological parameters affecting the biodegradation of sorbed- and separate-phase contaminants in soil-water systems is admittedly complex and presents an investigator with numerous experimental challenges. The analysis of the data reported from both natural and engineered systems clearly shows that the results obtained by any individual investigator are highly dependent on the target substrate being examined, the identity and concentration of the organism(s), and the nature of the sorbent. To date, there seem to be few common principles that govern the rate of degradation of a selected pollutant. However, the diversity of the sorbent-water systems examined and the variations in experimental design employed by investigators renders the existing studies virtually impossible to compare. Models to predict experimental observations are sophisticated in their approach to describing mass transfer kinetics yet are simplistic in their approach to describing biodegradation and cell growth."

Thus, ambiguity is the rule when examining the influence of solid surfaces on microbial activity and the relationship between bioavailability and biodegradation in soils. It is within this established, yet confusing, scholarly context that this report aims for progress.

8.2 Selection and Justification of Criteria for Identifying the Highest Quality Investigations Pertinent to Bioavailability and Bioremediation

This report has been designed to equip a reader with tools to decipher a vast and ambiguous literature on bioavailability and biodegradation. Previous sections of this report serve as a prelude that establishes the foundations for this eighth section: Section 2 defined bioremediation and how both microorganisms and plants can influ-

ence soil contaminants; Section 3 defined bioavailability; Section 4 surveyed results of bioremediation field projects; Section 5 examined mechanisms of persistence, as illustrated especially by soil organic matter; and Sections 6 and 7 of this report have provided a glimpse into what is and is not known about geosorbent-contaminant interactions and the mechanisms by which microorganisms and plants acquire contaminants in soil. This section of the report directly addresses the relationship(s) between the bioavailability of chemical wastes in soil and their bioremediation.

Previous sections of this report have emphasized the multifaceted nature of "bioavailability." It is not an inherent property of substances under examination. Rather, it reflects the response of experimentally defined biological systems to many integrated processes. Bioavailability is an emergent, malleable trait that is inferred from detailed, three-way interactions between geosorbents, biota, and chemical wastes. The "malleable" aspects of bioavailability stem from the fact that when scientific investigators design their experiments, intentional or unintentional choices are made. Each choice influences the outcome of the experiments. Which geosorbent will be used (e.g., model system or environmental sample, agricultural or industrial soil, freshwater sediment, of high organic matter or high clay, contaminated or pristine)? Which chemical waste will be studied (e.g., organic or inorganic; soluble or insoluble; volatile; toxic; hydrophobic or hydrophilic, freshly added or historically in place)? Which of the many possible biota (e.g., pure microbial cultures or mixed cultures, monocotyledonous or dicotyledonous plants, soil enrichments, microbial communities from contaminated or pristine soil) will be included? How will the geosorbents, chemical wastes, and biota be pretreated, mixed, and incubated? Are the experimental choices for system components and incubations realistic? How environmentally significant are the incubation conditions, the biota, and the physical/chemical states of the contaminants? What artifacts may be created in the data-generating systems or by the experimental designs and treatments? Can broad, sweeping principles and conclusions be drawn from particular sets of specific, narrowly defined experiments?

The above types of questions and their answers begin to impinge on philosophical issues about the scientific method and what it can achieve. These philosophical concerns are clearly beyond the scope of this report. Nonetheless, based on the literature reviews cited in Section 8.1, it is clear that, to some degree, the outcome of experiments examining relationships between bioavailability and biodegradation are arbitrary—virtually any outcome can be achieved when particular combinations of geosorbent, contaminant, and microorganisms or plants are selected. Given this possibility, interpretation of the multitude of reports must be grounded in the following characteristics:

(1) realism and environmental relevance,

(2) absence of experimental artifacts, and

(3) consistency of results.

Without such a foundation, many of the existing studies on how bioavailability influences biodegradation provide confusing or misleading results. Moreover, these criteria should help identify investigations whose results apply to society's real-world problems in real-world situations.

Table 2 lists the three characteristics selected for evaluating reports to be scrutinized in this section of this report (see Section 8.3 and Table 4). To identify investigations of the highest quality (realism, absence of artifacts, consistency; first column of Table 2), seven specific criteria for evaluating experimental procedures will be applied (middle column, Table 2). The first criterion is that the investigation begin with field samples and field observations of the persistence of contaminants. This guarantees the environmental relevance of a study. The second criterion recognizes that hypothesis testing to explain field persistence often requires manipulation of geosorbents and/or biota in the laboratory—but this should be done in a realistic manner, with minimal departure of experimental system from field conditions. The third criterion (realistic biota) recognizes that the microbial world is extremely diverse and responsive to laboratory-imposed conditions (Madsen, 1996, 1998), thus, misleading assays of biodegradation and/or bioavailability may occur if the biotic component of experimental systems is unlike that found in real-world settings. The fourth criterion acknowledges that exploring the basic physiological, biochemical, and genetic details of biological processes requires the use of single organisms. This is the major strength of pure-culture studies. Yet, single-organism studies need to be tempered and interpreted with realism—extrapolation from pure cultures in the laboratory to unknown complex microbial communities is usually unwise.

The seventh criterion in Table 2 simply expects that meritorious investigations build on previous results in a way that clearly makes progress toward answering significant scientific and technological questions. Given the many independent variables that must be confronted in biodegradation and bioavailability studies (e.g., Sections 3.1, 6.1, 8.1), discrepancies should be expected; nonetheless, the validity of results from studies that contradict one another (especially if generated by the same laboratory) should be questioned and not be considered of the 'highest quality."

Entries 5 and 6 in the central column of Table 2 describe criteria for reducing the credence given to biodegradation/bioavailability investigations based on artifacts that may be imposed on the geosorbent matrix under study. This is, admittedly, a controversial issue because compromise is often unavoidable in experimental procedures. If an experimental method is imperfect, do the imperfections erase all validity of the results? The answer is "probably not." Nonetheless, it is indisputable that the physical, chemical, and biological status of geosorbent matrices drastically influence the results of biodegradation and bioavailability assays occurring therein. Therefore, for the purposes of this review, it is desirable to judge the highest-quality investigations as those that avoid drastic alteration of the geosorbent matrix. Useful, supportive, confirmatory information can certainly be generated from

TABLE 2 Criteria for Identifying the Highest Quality Investigations Pertinent to Bioavailability and Bioremediation (See Section 8.2 for Rationale)

Desired Characteristic	Criteria Applicable to Bioavailability/Biodegradation Reports	Rationale
Realism and environmental relevance	1. Initial data should be obtained from field observations of contaminants	1. There is such an abundance of real-world contamination problems, that contrived, laboratory fabrications of contamination scenarios may be superfluous.
	2. Subsequent (e.g., laboratory-based) data should be obtained without drastic chemical or physical alteration of geosorbents or contaminants	2. After documenting the field behavior of contaminants, hypothesis testing may need to occur via sample incubation in the laboratory. When brought in from the field, neither the geosorbents nor contaminants should be unduly altered, unless warranted by experimental objectives.
	3. Biota used in bioassays should represent those that are native to the field site	3. When the experimental objective is to reveal biodegradation and/or bioavailability characteristics of real-world soil microbial communities, then appropriate, representative biota should be used in the experiments.
	4. When pure cultures are examined, their relevance and the experimental conditions selected (such as physiological status) should be defended for their environmental relevance	4. If refined hypotheses warrant examination of pure microbial cultures, then care should be taken to relate experimental variables of pure-culture manipulations back to specific hypotheses and their relevance to field conditions.
Absence of experimental artifacts	5. Avoidance of soil sterilization procedures	5. Chemical and physical treatments that allow sterile (abiotic) controls to be examined drastically alter the properties of geosorbents.

TABLE 2 Criteria for Identifying the Highest Quality Investigations Pertinent to Bioavailability and Bioremediation (See Section 8.2 for Rationale) (Continued)

Desired Characteristic	Criteria Applicable to Bioavailability/Biodegradation Reports	Rationale
	6. Avoidance of contaminant addition via flash evaporation from volatile carrier solvents	6. When contaminants are artificially added to geosorbents in highly volatile organic solvents, the rapid evaporation of the solvent may leave the contaminants in a physical/chemical state and/or location that is unlike that of contaminants in field soils. Also, carrier solvents are likely to remain in the geosorbents, unless they are subject to extensive measures that encourage evaporation.
Consistency of results	7. Data from one set of experiments should support and not conflict with another subsequent similar set of experiments	7. Although variability in physical, chemical, and biological properties of real-world samples has become an accepted means of "explaining" conflicting experimental results, resorting to such excuses is undesirable. Unresolved inconsistency may detract from understanding biodegradation and bioavailability issues.

sterile (entry #5) or spiked (entry #6) geosorbent matrices—but in this report, such investigations will be considered secondary, not primary, information sources.

8.2.1. Further Scrutiny of Artifacts That May Be Caused by Soil Sterilization and Contaminant Addition in Organic Solvents

Soil sterilization (entry #5, central column of Table 2) is required for the preparation of abiotic controls of many experimental designs (Brock, 1978). Yet "sterilization procedures result in some degree of alteration of soil chemical and physical properties" (Wolf and Skipper, 1994). Measures that have been used to produce abiotic controls include chemicals that inhibit microbial metabolism (e.g., acid, azide, mercuric chloride) and sterilization (e.g., by autoclaving or γ-irradiation). All of these treatments do far more than simply eliminate biological activity. Cawse (1975) summarized much of the early literature on the influence of autoclaving and γ-irradiation procedures on soil properties and the activity of reinoculated microorganisms. Influences include: solubilization of organic matter, release of electrolytes, formation of inhibitory substances, lower pH, and many detailed alterations in the chemical composition of soil solution. In preparing abiotic controls using low organic matter subsurface sand, Ball (1989) found that overnight heating or overnight cooling within an autoclave tended to increase the sand's sorptive properties, but the effect could be avoided if the sand samples were cooled rapidly after autoclaving. Autoclaving of this same sand also was found to increase its affinity for binding hexachloroethane (Criddle *et al*., 1986; MacKay *et al*., 1986). Xia (1998) also observed varying effects of autoclaving on phenanthrene sorption by several different subsurface materials. In a study designed to address PAH bioavailability in soils, Sandoli *et al*. (1996) discovered that γ-irradiation-induced changes in geosorbent properties prevented ^{14}C-phenanthrene mineralization in the presence of otherwise active microorganisms. No evidence for toxicity was evident in these γ-irradiated soils. Although gamma irradiation was found to increase the geosorbent's affinity for phenanthrene by 32 percent, a full explanation of the γ-irradiation-induced attention of the geosorbent was not obtained (Sandoli *et al*., 1996). Unfortunately, the impacts of inhibitors and sterilization procedures on physical, chemical, structural, and sorptive properties of soils are probably as variable as the properties of soils themselves.

Discrepancies in the properties of "freshly added" versus "field contaminated" pollutants in geosorbent matrices have been noted in many investigations (e.g., Alexander, 1999; Burford *et al*., 1993; Hatzinger and Alexander, 1995; Steinberg *et al*., 1987). However, seldom have the two distinctive issues pertinent to these discrepancies been discussed. The issue that has perhaps received greatest attention to date is that the physical, chemical, and biological behavior of freshly added contaminants may change over time. Therefore, freshly added contaminants may not mimic the behavior of "aged material." It has been postulated that the freshly

added contaminant pool may not have had sufficient time to diffuse into micro- or nano-pores or be absorbed into organic matter (e.g., Alexander, 1995; Luthy *et al*., 1997; Reid *et al*., 2000). Thus, an "aging effect" has been used to argue for kinetically constrained, time-dependent sequestration of environmental contaminants.

The second related but distinctive issue for freshly added contaminants (entry #6, central column of Table 2) is that the way they are added to geosorbents may leave them in locations and in physical/chemical states that differ markedly from their field-derived analogs. When small volumes of contaminant-containing volatile solvents are added ("spiked") to soil and allowed to evaporate, then crystals of the contaminant solute may be left behind in the soil matrix. It is unlikely that these crystals, even if later distributed uniformly throughout the soil via mechanical mixing, will initially interact with the soil matrix in ways that mimic the interactions found in field sites (unless the field sites happen to be contaminated via similar flash-evaporation-type procedures; S. Hawthorne, personal communication). Not only are the flash-evaporated spiked contaminants likely to be in states and associations and locations unlike those in the field, but complete removal of the carrier solvent requires heat and vacuum because the solvent's effective boiling point can be raised in small pores (J. Pignatello, personal communication). Without such directed effort to remove residual solvents from spiked soil, they may have unanticipated effects on soil organic matter (e.g., swelling that may be irreversible; J. Pignatello, personal communication), contaminants, and/or microbial processes. Burford *et al*. (1993) carried out an extensive comparison of the extraction efficiencies of freshly added, deuterated PAHs with corresponding nondeuterated PAHs from historically contaminated soil and sludges. Burford *et al's*. (1993) data consistently showed that freshly added PAHs (dissolved in dichloromethane and flash evaporated) were far more extractable than the native PAHs. The discrepancy between freshly added and long-aged PAHs was greatest for the low molecular weight PAHs (e.g., naphthalene). Burford *et al*. (1993) concluded that "it is experimentally impossible to reproduce the environmental conditions that occur during deposition of pollutants in real-world samples."

This comment by Burford *et al*. (1993) is particularly germane to the interpretation of data describing "aging" of contaminants in geosorbents. Flash-evaporated surrogate contaminants may be dispersed in laboratory-incubated soil experiments. However, once in place, the contaminants will undergo dissolution, diffusion, partitioning, and other reactions governed by a combination of contaminant properties, the soil, and the incubation conditions. The many investigations describing "aging effects," noted above, have documented time-dependent alteration in biological and/or chemical properties of the contaminants. These investigations have not shown that the time-dependent (perhaps asymptotic) change approaches the same endpoint that is found in field-contaminated soils. After all, the real-world weathering processes (water infiltration, freeze/thaw, wind, sunlight, etc.) that occur in uncontrolled field sites are vastly different from the typical conditions found in laboratory incubations. It is

possible that contaminants spiked with geosorbents and subsequently aged in the laboratory eventually attain the same physical, chemical, and biological properties as those found in the field. Indeed, "field contaminants" probably occur in a broad spectrum of states, and are almost certainly neither uniform nor static. **However, it is reasonable to presume that both the rate at which the "field state" is asymptotically approached, and the asymptote itself, are influenced by the condition of the geosorbent and the incubation. To the degree that these two variables deviate from real-world conditions, the state of laboratory-aged contaminants also may deviate from those found in real-world soils. Currently, there is not enough information addressing the above-described uncertainties about sterilization, spiking, and aging procedures and their impact on contaminants in geosorbents. Until the relationships between field- and laboratory-contaminant disposition in soils are better understood, it will remain a challenge to interpret laboratory-based experiments that attempt to test hypotheses about field processes using sterile, spiked soils.**

8.3 Scrutinizing Selected Investigations Describing the Bioavailability of Contaminants and Their Biodegradation

To some degree, M. Alexander has become an advocate for incorporating bioavailability considerations into regulatory and/or toxicological interpretation of environmental contamination. Therefore, it seems appropriate to examine and scrutinize his recent, relevant publications. In 1995 and 1997, Alexander published critical reviews that advanced the idea that contaminant bioavailability in soil may be the most relevant criterion for "assessing toxicity, determining risk, and establishing meaningful regulations for clean up of sites containing hazardous wastes." Alexander presents six lines of evidence to support the proposition that organic compounds become increasingly sequestered (less bioavailable) as their residence times in soils increase. These lines of evidence appear in Table 3, along with alternative interpretations of the "evidence." The alternative interpretations (second column) for evidence presented in the first column of Table 3 simply apply the background knowledge and principles that have been established in earlier sections of this report. Although all of the lines of evidence are plausible, they also need to be carefully reexamined for the possibility of alternative interpretations and also for the quality of supporting experimental data. As indicated in the second column of Table 3, there are many potential weaknesses in the experiments supporting the six lines of evidence, including: (1) many factors besides diminished bioavailability can contribute to the long-term persistence of pesticides in soils; (2) observations in the field combined with laboratory experiments (using soils that have been contaminated for extended periods to examine the cause of contaminant persistence) may be the most robust type of evidence, given the quality of the data; (3) laboratory aging studies may be rich in artifacts, especially if sterile, spiked soils are used (see Section 8.2.1); (4) chemical extraction experiments that clearly demonstrate distinctions between aged and freshly contami-

TABLE 3 Lines of Evidence for Time-Dependent Decline in Bioavailability of Organic Soil Contaminants (from Alexander, 1995, 1997) and Possible Alternative Interpretations

Evidence	Alternative Interpretations
(i) A "hockey stick"-like profile of concentration versus time for pesticides in field soils. The initial rapid loss of pesticides, followed by a plateau, suggests increased sequestration over time.	(1) Most data were from chlorinated pesticides that are not readily biodegradable because they do not serve as carbon and energy sources for microorganisms. Alternative hypotheses for explaining persistence of pesticides (such as lack of cosubstrate, simple first order kinetics, nutrient limitations, other limiting environmental factors) were discussed in Sections 4.0 and 5.2. Without considering all alternative hypotheses, the "bioavailability" hypothesis cannot be adequately tested.
(ii) Laboratory studies of field samples contaminated with pesticides and PAHs in which native soil microorganisms were able to biodegrade a freshly-added, but not an "aged" pool of these compounds.	(ii) This evidence can be very convincing (see Table 4)
(iii) Laboratory "aging" studies in which the degree of biodegradation by a microbial inoculum was governed, inversely, by the duration of prior contact between organic chemicals and sterile soil.	(iii) Conditions for laboratory "aging" may be artifactual, especially if sterile soils and contaminants are added via flash-evaporated organic solvents (see Section 8.2).
(iv) Chemical extraction assays showing that the degree of sequestration of organic compounds in soil is proportional to the duration of compound-soil contact.	(iv) This evidence may or may not be convincing (see Section 8.2.1). A change *per se* is not adequate. The change in contaminant extractability must be toward an environmentally relevant endpoint.
(v) Analysis of the kinetics of contaminant desorbtion from soils that suggested the presence of recessed diffusion-limited binding sites within pores of soil particles or within organic matter phases.	(v) This evidence may or may not be very convincing. The test systems are highly complex and variable, hence, susceptible to many alternative interpretations (see Sections 6.3 and 6.5). Validity depends on details of specific procedures in specific investigations.

TABLE 3 Lines of Evidence for Time-Dependent Decline in Bioavailability of Organic Soil Contaminants (from Alexander, 1995, 1997) and Possible Alternative Interpretations (Continued)

Evidence	Alternative Interpretations
(vi) Bioassays showing that chemicals residing in soil have reduced toxicity and this reduction may be proportional to the chemical's soil residence time.	(vi) This evidence may or may not be very convincing. The test systems are highly complex and variable, hence, susceptible to many alternative interpretations (see Sections 6.3 and 6.5). Validity depends on details of specific procedures in specific investigations, the bioassay, and the specific mechanism of toxicity.

nated soils are insightful, though scrutiny of spiking and other procedures is warranted (see Section 8.2); (5) macroscopic observations of the interactions between contaminants and geosorbents have many varied conceptual interpretations (Sections 6.1 and 6.5); and (6) diminished contaminant toxicity in the presence of soil solids has many alternative explanations, depending on the bioassay, the soil, and the mechanism of toxicity.

The quality of experimental data (e.g., field observations, type of model systems, potential experimental artifacts; general conformity to the seven criteria in Table 2) is of crucial importance in evaluating all "lines of evidence" for or against a reduction of contaminant bioavailability in soils. Another major consideration is whether or not "statistically significant" differences between experimental treatments reported in the peer-reviewed literature have real-world ramifications. Baveye and Bladon (1999) pointed out that small, though statistically significant short-term treatment differences, may be moot when extrapolated to real-world timeframes of months to years.

A final, key element to include is provided by an organic substance whose persistence is well established—soil organic matter. How can the behavior of soil organic matter enlighten our understanding and expectations for the behavior of chemical wastes in soil?

Table 4 contains a compilation of some of the most influential and/or recent experimental studies that have been published to date on the relationship between bioavailability of organic compounds and bioremediation. The columns in Table 4 (Goals, Compound, Geosorbent, Approach/Methods/Data, Conclusions, Methodological Weaknesses) provide a format to prioritize and evaluate a study using the criteria established in Table 2.

The first entry in Table 4 (Steinberg *et al*., 1987) is perhaps the most convincing study to document the relationship between an aged organic chemical waste and its availability for bioremediation. Unlike the majority of entries in Table 4, the study by Steinberg *et al*., (1987) suffers from no obvious methodological weaknesses—it satisfies all seven criteria in Table 2 for high-quality data. Steinberg *et al*. (1987) began with field observations that the fumigant, ethylene dibromide (EDB), had persisted in field soils for an unexpectedly long time. Subsequent laboratory experiments used chemical, physiological, and physical measurements to prove that aged, field-applied EDB was not biodegraded by the native soil microbial community; yet, freshly added radiolabeled EDB was readily metabolized. Furthermore, measurements of the rate of release of the aged EDB from soil revealed that it was kinetically retarded and that release could be accelerated by pulverizing the soil. Entrapment of EDB in soil micropores was inferred. The study by Steinberg *et al*. (1987) did not use sterile soils or flash-evaporated "spiked" soils. Moreover, the study was thorough, internally consistent, and provided convincing data supporting logical arguments for a causal relationship between diminished bioavailability and diminished biodegradation.

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
1. Explain anomalous persistence of volatile, biodegradable pesticide in field soils	1,2-Ethylene Dibromide (EDB), a gas phase-applied fumigant with aqueous solubility of 3,370 ppm	Four tobacco farm soils treated with EDB in the field 0.9 to 19 years prior to analysis	Use chemical and microbiological assays to assess if long-aged (native) EDB was distinctive from freshly added EDB Freshly added EDB was volatile; aged EDB was not Aged EDB did not readily equilibrate with aqueous solution. Rates of release of aged EDB from soil into aqueous solution were temperature dependent. Diffusion-based modeling of EDB release to aqueous solution suggested 2-3 decades to reach 50 % of predicted equilibrium values.	Residual EDB was highly resistant to both mobilization and microbial degradation There is slow exchange between "native," aged EDB and the freshly added chemical Pulverization of the soil drastically accelerated chemical release of EDB from soil Entrapment of EDB in micropores was inferred		Steinberg *et al.*, 1987

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			The biodegradation tests showed that freshly added ^{14}C-EDB was readily converted to a combination of $^{14}CO_2$ (46 %) and cell material (54 %), while the long-aged EDB was not			
2. Construct, operate, and manage a field-scale, prepared bed, land treatment unit for 2 soils; assess performance based on contaminant mobility, toxicity, concentration; determine treatment to	Crude oil (aged in field 30 to 40 years)	Clay soil from Texas gas storage facility	Use soils aged in the field; treat with tillage, nutrients, water (clay soil, 55 weeks or silty sand, 34 weeks) In clay soil, long contaminated with crude oil, total petroleum hydrocarbons (6,400 to 11,000 ppm) showed no decline and leachability declined from 4.4 to 1.3 ppm	After field treatment, laboratory incubations (in which oxygen and nutrients were added and viable petroleum-degrading microbial populations were found) failed to stimulate biodegradation This, and absence of inhibitory salts or metals, suggests that bioavailability		Olivera *et al.*,1998

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
reach environmentally acceptable endpoint			total petroleum hydrocarbons (TPHs). Field leachate ranged from 0.1 to 75 ppm, and only a trace of toxicity (microtox assay) was found	limited microbial activity		
	Diesel fuel (aged in field 3 years)	Silty sand from Texas power plant	In the silty sand contaminated with diesel fuel, TPH declined from 1,100 to 160 ppm, aqueous release (TCLP) ranged from 1.5 to 3.2 ppm, field leachate ranged from 1.1 to 7.8 ppm, and only a trace of toxicity was found.	"Active, prepared bed treatment of about 15 weeks reached an environmentally acceptable endpoint"		
3. Use laboratory protocols to quantify	Crude oil (aged in field 30 to 40 years)	Clay soil from Texas gas storage facility	Build on results of Olivera *et al.* (1998) by using laboratory	No degradation of hydrocarbons occurred, nor could		Berg *et al.*, 1998

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
rate of release of petroleum hydrocarbons; investigate impacts of bioremediation on hydrocarbon release from soils; explore possibility that bioremediation is limited by hydrocarbon release			measurements of the rate of release (ROR) of petroleum hydrocarbons from bioremediated field oils and their residual fraction The ROR procedure used XAD2 resins as a constant sink for contaminants in long-term aqueous-phase desorption studies. The released contaminants were subsequently analyzed by GC/MS	releases of hydrocarbon be quantified; "Lack of biodegradation was related to inability of chemicals to be released from the soils" "This indicated low bioavailability of hydrocarbon"		
	Diesel fuel (aged in fields 3 years)	Silty, sand from Texas power plant		Bioremediation by land treatment diminished the mass of hydrocar-	Data analysis yielded some anomalous (impossible)	

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
				bons released from soil; an initial rapid release was followed by a slow-release phase	modeling parameters from the contaminant release curves	
4. Determine the limits and extent of hydrocarbon biodegradation toxicity and leachability in petroleum contaminated soil	Three different weights of crude oil, artificially weathered at 5 % (wt/vol) freshly added	Two soils, one low, one high in organic matter, initially uncontaminated	Implement an 8–11 month bioremediation treatment (of moist, nutrient-amended soil) and then perform chemical analyses, toxicity, and leachability assays. Depending on soil, weight of oil and hydrocarbon chain length, between 10 % and 88 % of the initial oil was biodegraded. BTEX was almost always below detection	Biotreatment reduced mobility and eliminated toxicity of residual hydrocarbons Residuals were not toxic or leachable, or biodegraded further	Both contamination and bioremediation events were conducted in the laboratory	Salanitro *et al.*, 1997

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			Biotreatment caused drastic reduction in leachable BTEX, eliminated toxicity to earthworms, and diminished inhibition of seed germination Plant growth assays of toxicity revealed stimulation by oil			
5. Test the hypothesis that slow desorption is the cause of limited biodegradation; and if this is the case, investigate prediction of bioremediation	Fifteen PAHs	Two PAH-contaminated sediments from Amsterdam Harbor	Desorption kinetics of 15 PAHs were determined before and after biotreatment via bioreactors (4 months) or land farming (2 years) Desorption kinetics were measured by trapping on Tenax resins Biotreatment	For readily metabolized PAHs, the extent of possible PAH degradation could be roughly predicted from the initially desorbing fractions However, a pool of PAHs was desorbed but not		Cornelissen *et al*., 1998

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
based on desorption kinetics			reduced the rapidly desorbing PAH-fraction A pool of non-degrading PAHs also desorbed; but this pool remained unchanged by biotreatment	metabolized, probably due to microbial factors, not lack of bioavailability		
6. Test hypotheses for explaining the long persistence of naphthalene in sediments	Naphthalene (sparingly soluble, 31 ppm)	Sandy, organic matter-rich surface sediments	Use chemical and microbiological assays to attempt to identify factors in field that prevent biodegradation; GC analysis of field samples, $^{14}CO_2$ production from ^{14}C- naphthalene added to laboratory-incubated samples;	In laboratory incubations, naphthalene metabolism was oxygen-limited, yet H_2O_2 addition in the field did not stimulate naphthalene loss *in situ*; no evidence for nutrient limitation was obtained; soil	γ-irradiated soils were used in the aging study; the laboratory aging period was relatively short (4 weeks); chemical analysis of field samples introduced much variability in an attempt to	Madsen *et al.*, 1996

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			amendment of sample, with nutrients, aging of γ-irradiated soils followed by mineralization assays, addition of H_2O_2 to field sediments followed by GC analysis	slurries enriched in sorbed naphthalene metabolized the aged substrate; no clear evidence to distinguish oxygen limitation from sequestration as the cause of persistence	document biodegradation *in situ*; different enrichment cultures reacted differently to aged substrates	
7. Compare bioavailability and desorption kinetics of weathered (aged) and recently added herbicide	Simazine (chlorinated triazine herbicide that is not a microbial carbon and energy source; sparingly soluble in water, 3.5 ppm)	Agricultural soil receiving simazine annually for more than 20 years	Sorption coefficient for aged simazine was 15 times that of freshly added; simazine concentration in soil solution in field soils showed that the aged material was far below aqueous equilibrium concentration; freshly added simazine was toxic to sugar beet	Pesticide aging diminished bioavailability, as measured by microbial degradation and plant uptake Desorption kinetics of aged simazine were slow compared to the recently added chemical	Investigators did not clearly describe the means by which biodegradation of freshly added ^{14}C-simazine was discerned from the total simazine pool Sorption coefficients for ^{14}C simazine were measured with	Scribner *et al.*, 1992

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			seedlings, aged simazine was not		DMSO as carrier for the simazine (though simazine soil solution data were genuine, carrier-free, field measurements)	
8. Determine the effect of aging time in soils on the biodegradability and extractability of organic compounds	Phenanthrene (sparingly soluble 1.3 ppm) and 4-nitrophenol (readily soluble in water)	3 soils of varying properties (agricultural soil, muck soil, aquifer sand)	Using γ-irradiated, sterile, spiked soils, the contact time between geosorbent and the two ^{14}C-labeled test contaminants was varied between 0 and 315 days; then pure cultures of bacteria were inoculated into the geosorbent and $^{14}CO_2$ evolution from the contaminants was measured; phenanthrene was	"The data show that phenanthrene and 4-chlorophenol added to soil become increasingly more resistant with time to degradation and extraction." Rates and extents of mineralization were inversely proportional to the aging duration	γ-irradiation of soil; phenanthrene contaminant was added in an organic solvent; pure cultures were used in biodegradation tests	Hatzinger and Alexander, 1995

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			spiked into soil using dichloro-methane as a carrier, 4-chlorophenol was added in water; simultaneous to $^{14}CO_2$ evolution assay, the labeled compounds were extracted from soil using butanol and soxhlet procedures; sonication of soil aggregates slightly enhanced mineral-ization			
9. Use a nonsterile soil system to assess biodegradabil-ity (bioavail-ability) and extractability of differen	Phenanthrene	Soil from Sierra Nevada foothills	Age (0 to 600 hours) phenan-threne in a nonsterile soil with little biodegradation activity, then add active inoculum, and analyze $^{14}CO_2$ evolution kinetics,	Both the hexane extracts and mineralization curves showed a rapid (200 h) de-cline in the availability of phenanthrene; mild extraction	Short, aging periods; phenan-threne was added in methylene-chloride organic solvent	Schwartz and Scow, 1999

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
tially aged phenanthrene			as well as phenanthrene extractability	procedures reflect bioavailability; sorption processes may prolong the presence of a chemical in soil		
10. Examine competitive displacement of aged phenanthrene by pyrene in chemical extraction and biodegradation assays	Phenanthrene	Agricultural soil and peat soil	Mineralization and chemical extractability of aged radiolabeled phenanthrene (3 to 123 days) in two sterile soils, with and without added pyrene were measured Soils were sterilized by γ-irradiation; ^{14}C-phenanthrene was coated onto the wall of a flask and then allowed to equilibrate through the aqueous phase with soil.	Aging diminished rate and extent of mineralization; pyrene addition enhanced phenanthrene mineralization; phenanthrene extractability and K_d values also were altered by pyrene; the pyrene influences may have been caused by competitive displacement of the phenanthrene from glassy organic matter	γ-irradiation of soil; a pure culture was used in biodegradation tests	White *et al.*, 1999

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			Pyrene was added in methanol as a carrier (<0.1% by volume)			
11. Model coupled processes of desorbtion and mineralization using two distinctive model organisms	Naphthalene (a sparingly soluble hydrophobic compound; 31 ppm)	Agricultural soils—dried, ground, and autoclaved	Naphthalene mineralization by two different bacterial cultures was examined in aqueous solution containing varying amounts of added soil	For one bacterium, sorption limited the rate and extent of slurry-phase naphthalene mineralization. This was not the case for the other organism, which metabolized a significant portion of sorbed naphthalene	Soils were air-dried, ground, and autoclaved. ^{14}C-naphthalene was added to soil slurries in acetone as initial carrier; pure cultures were examined	Guerin and Boyd, 1992
12. Determine how microbial activity influences associations between soil organic matter and	Pyrene (solubility 0.13 ppm in water)	A forest soil and an inoculum from a pyrene-contaminated Superfund site	The distribution of ^{14}C- pyrene and byproducts were determined by sequential soil extraction procedures from forest soil, with or without a pyrene-	Over time, the extractability of pyrene and pyrene products decreased to a greater extent in the metabolically active soil Biological activity	Methanol was used as the pyrene solvent It is difficult to interpret the meaning of extractability changes in such a	Guthrie and Pfaender, 1998

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
contami-nants			degrading inoculum or a metabolic inhibitor. Bioavailability was assessed by $^{14}CO_2$ evolution from radiolabeled pyrene added to soil in constantly flushed incubation chambers	may reduce biotoxicity. The amount of $^{14}CO_2$ released from freshly added and aged (270 days) pyrene was similar	complex experimental system	
13. Describe the potential release and subsequent degradation of bound ^{14}C residues from soil previously treated with atrazine	Atrazine, water soluble chlorinated triazine herbicide that was not a ready carbon and energy source for soil micro-organisms	Agricultural soil	Form ^{14}C-atrazine bound residues in soil via a 1-year laboratory incubation; exhaustively extract with methanol to remove residual ^{14}C; chemically characterize the residual ^{14}C in soil; inoculate the soil with two different atrazine-degrading	During an 84-day incubation, atrazine and four metabo-lites were released from the initially soil-bound ^{14}C atrazine; different bacteria released hydroxyatrazine to different extents; soil bound residues can be released	Soil sample prepared by exhaustive extraction with methanol; use of pure cultures	Khan and Behki, 1990

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			bacteria with and without a glucose amendment; assess metabolism of the bound ^{14}C			
14. Examine the fate of soil-aged nonextractable atrazine with respect to biodegradation, microbially facilitated release, and abiotic desorption	Atrazine (water soluble)	Agricultural silt loam	After 3 months of aging atrazine in sterile soil, the extractable atrazine was removed. Next, the soil containing residual atrazine was subjected to mineralization and desorption tests Almost no (<2.5%) atrazine mineralization occurred Viable microorganisms did not enhance release of previously bound atrazine	Bound residues are subject to desorption and release from soils Rates of atrazine mineralization were exceeded, in nearly all cases, by the desorption rates—suggesting no mass transfer limitations	γ-irradiated soils were used to age (bind) the atrazine in soil Test substrate (atrazine) was not appreciably metabolized, thus the bioavailability assay was not robust	Johnson *et al.*, 1999

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
15. Compare biodegradation of sorbed- and solution-phase compound in soil	2,4-D (a reasonably water soluble compound; 900 ppm)	Two agricultural soils and one clay, all sterilized by autoclaving	Three separate mathematical models were used to evaluate the mineralization of 2,4-D added to soil and metabolized by an inoculated pure culture	The model that best fit the data presumed that sorbed 2,4-D was unavailable	Soils were autoclaved and ground; a pure culture was used; 2,4-D was freshly added to soil with methanol as initial carrier. A 5-h incubation time was used	Ogram *et al*., 1985
16. Assess the feasibility of using chemical extraction procedures to estimate bioavailability of pesticides to earthworms	DDT, DDE, DDD (sparingly soluble chlorinated insecticide or byproduct)	Seven agricultural soils of varying properties and pesticide application histories	Determine pesticide concentrations via soxhlet extraction/ analysis; measure earthworm uptake of pesticides; correlate earthworm uptake with C18 membranes and 25% tetrahydrofuran extracts; age pesticide (0 to 924 d) in γ-irradiated soils; pesticides were	Worms assimilated 3% to 66% of the pesticides; this uptake correlated well with chemical extraction assays. Correlation coefficients were 0.921 or higher for the C18 membrane and 0.83-09.48 for the tetrahydrofuran extraction	γ-irradiation of soil; addition of pesticides in organic solvent; incompletely vented	Tang *et al*., 1999

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			added in dichloromethane solvent allowed to volatilize for 48 h			
17. Assess the decline in bioavailability as a result of aging of pesticides	DDT, DDE, DDD, dieldrin	Three agricultural soils of varying properties and pesticide application histories	Use an earthworm uptake bioassay to measure pesticide bioavailability in field soils and laboratory-aged soils; pesticide-uptake assays involved exposing worms to soil, then harvesting, extraction, and GC analyses; field aged soils (49 and 30-year periods) were used; laboratory-aged soils (0, 90, 190 days) were γ-irradiated and pesticides were	Aging materials reduces bioavailability Soil extraction by Tenax resin correlated well with earthworm uptake for three of four pesticides	γ-irradiation of soil; addition of pesticides in organic solvents, incompletely vented In many instances, the data showed that aging had no effect on bioavailability	Morrison *et al.*, 2000

TABLE 4 Survey of Laboratory and Field Studies Aimed at Discerning Mechanistic Relationships Between the Biodegradation of Organic Compounds and Their Bioavailability in Soil (Continued)

Goals	Compound	Geosorbent	Approach/ Methods/Data	Conclusions	Methodological* Weaknesses	Reference
			added in organic solvents; chemical analysis of the pesticides adsorbed to Tenax resin was examined as a possible surrogate for earthworm uptake			

* Departure from the 7 (seven) quality criteria in Table 2

The next two entries in Table 4 were conducted by R. Loehr's research group at the University of Texas. Like Steinberg *et al*. (1987), a field approach was taken, and subsequent hypotheses were sequentially tested to try to explain the persistence of organic soil contaminants subjected to bioremediation processes; no methodological weaknesses could be discerned. For Olivera *et al*. (1998) and Berg *et al*. (1998), the contaminants were petroleum hydrocarbons (crude oil and diesel fuel) that had been in the field for either 3-4 decades or 3 years before being field bioremediated (land treatment) for either 55 or 34 weeks. Chemical analysis of the field-bioremediated soils was used to monitor process effectiveness. Simultaneously, the mobility (leachability) of the residual hydrocarbons and their toxicity were measured. The behavior of the different hydrocarbons (aged in two different field soils for different periods) was not uniform: land treatment reduced residual hydrocarbons in the soil contaminated for 3 years, but not for 3 to 4 decades. Nonetheless, low hydrocarbon leachability and toxicity were documented for both soils. This suggested to the authors that "environmentally acceptable endpoints had been reached." Furthermore, alternative hypotheses for explaining the field persistence of the hydrocarbons (e.g., nutrient limitations, lack of viable microbial populations) were tested and dismissed. Thus, diminished bioavailability was implicated as the cause for persistence of hydrocarbons in biotreated field soils.

Another study addressing bioavailability and biodegradation of petroleum hydrocarbons in soils was that of Salanitro *et al.* (1997; entry number four of Table 4). The approach was to freshly contaminate soil with crude oil so that the naturally occurring microbial populations could biodegrade it. Then chemical analyses, as well as leachability and toxicity (earthworm and seed germination) assays, were performed to characterize the residual hydrocarbons. This study's only (minor) weakness was that it was done in a laboratory—it did not include true field observations of persistence or biodegradation activity. Nonetheless, Salanitro *et al's*. (1997) study met most of the criteria in Table 2 (real-world microbial populations, absence of artifacts possibly associated with sterilization and flash-evaporated spikes, and consistency). The conclusion seemed sound: concomitant with the persistence of hydrocarbon residuals in soils, the toxicity and mobility of petroleum hydrocarbons were substantially reduced by biotreatment.

Cornelissen (1998), the fifth entry in Table 4, gathered field-contaminated, polycyclic aromatic hydrocarbon (PAH)-rich sediments from Amsterdam Harbor, subjected them to bioremediation (either bioreactor or land farming), and measured desorption kinetics of the PAHs before and after biotreatment. This study had no obvious methodological weaknesses. It found that some PAH compounds persisted regardless of their desorption from sediments. However, for the biodegradable PAH pool, biotreatment was found to effectively degrade the otherwise rapidly leachable (releasable) PAHs. Cornelissen *et al*. (1998) concluded that the biodegradable PAH pool was predictable, based on the chemically measurable "rapidly desorbing fraction." This implied that the rapidly desorbing fraction was bioavailable and that the nondesorbing fraction was not.

Entry number six in Table 4 (Madsen *et al*., 1996) began with the goal of testing several hypotheses for the persistence of a readily biodegradable PAH compound, naphthalene, in a coal tar-contaminated study site. Cores removed from the site proved that naphthalene was present in anaerobic freshwater sediments. Furthermore, laboratory incubations proved that the native microbial community could readily convert freshly added ^{14}C-labeled naphthalene to $^{14}CO_2$ under aerobic conditions and that these microorganisms were not nutrient limited. The cause of contaminant persistence was narrowed to two hypotheses: lack of bioavailable naphthalene or lack of a final electron acceptor (O_2). To address the former hypothesis, irradiated sterile sediments were aged in the presence of ^{14}C- naphthalene and mineralization was assayed using inocula from the sediment. However, the maximum aging period was unrealistically short (4 weeks). In addition, two types of inocula (enriched on either solid- or liquid-phase naphthalene) responded differently to the aged naphthalene. Malleability of the microbial community was indicated because sediment microorganisms pre-enriched on soluble naphthalene did not easily mineralize the aged naphthalene, while unenriched sediment microorganisms mineralized aged and unaged naphthalene equally. To test the oxygen limitation hypothesis in the field, H_2O_2 was added to small volumes of enclosed sediments. However, over 3 weeks, the H_2O_2 treatment did not stimulate naphthalene loss from soil. Thus, Madsen *et al*.'s study (1996) was equivocal. Lack of field stimulation by the H_2O_2 amendment suggested a bioavailability limitation, but it was possible that the H_2O_2 treatment was ineffective. Similarly, the experiments using aged, irradiated sediments showed that diminished bioavailability associated with aging was dependent on the inoculum.

To understand field persistence of the herbicide, simazine, Scribner *et al*. (1992; seventh entry in Table 4) used chemical and bioassay methods to compare the partitioning behavior, biodegradability, and toxicity of freshly added versus aged simazine in soil. When simazine was freshly added in the field, it was found in the soil solution, but its fractional equilibrium concentration was otherwise very low in field pore waters. Moreover, freshly added, but not aged, simazine was both biodegradable and toxic to plant seedlings. This study presented laboratory data [much like that of Steinberg *et al*. (1987)] contrasting a constant pool of aged simazine that was not biodegraded in soil by the naturally occurring microflora with a declining pool of ^{14}C-freshly added simazine; however, the methods leading to such data were not clearly described. Overall, this study's results supported the view that an aged, field-applied pesticide was neither mobile nor bioavailable. The case was not flawless, however, especially because simazine is not a readily utilized carbon and energy source for soil microorganisms.

Hatzinger and Alexander (1995; entry #8 of Table 4) published a pivotal series of experiments that yielded clear, inverse relationships between the extractability and biodegradability of phenanthrene and 4-nitrophenol that had been aged in sterile (irradiated) soil up to 315 days prior to chemical and biodegradation analyses. Unfortunately, using the criteria established in Table 2, the work of Hatzinger and

Alexander (1995) has diminished merit because no field observations were made, sterile soil was used, the phenanthrene was added with a carrier organic solvent, and pure cultures were used in the mineralization bioassay.

The ninth entry in Table 4 (Schwartz and Scow, 1990) examined the behavior of a three-ring PAH compound, phenanthrene, which was added to a soil whose native microflora had unusually low phenanthrene biodegradation activity. After as long as 600 hours (25 days), chemical extractions and a phenanthrene-degrading microbial inoculum were used to assess the influence of aging on phenanthrene availability. Data showed that, although both the amount of hexane-extractable and biodegradable phenanthrene diminished with aging, the bacteria were able to access a pool of phenanthrene unavailable to the hexane extractant. Thus, some support for the diminished bioavailability of aged phenanthrene was garnered. But the results were somewhat ambiguous and the methods did not fully conform to the criteria of Table 2 (see column 6 of Entry #9 in Table 4).

A collaborative effort by laboratories operated by M. Alexander and J. Pignatello (White *et al.*, 1997; Entry #10, Table 4) sought to test hypotheses about binding sites for phenanthrene and how adding another PAH compound (pyrene) could influence phenanthrene's chemical and biological behavior. Aging was found to diminish both the rate and extent of phenanthrene mineralization. Moreover, the added pyrene was found to boost both extractability of the phenanthrene and its extent of mineralization by an inoculated bacterium unable to metabolize pyrene. Unfortunately, applying the criteria in Table 2, the work by White *et al.* (1999) was not field based and both irradiated soil and a pure culture were used in the experiments.

In 1992, Guerin and Boyd (entry #11 of Table 4) published an often-cited study illustrative of how pure cultures of bacteria can reveal contrasting physiological capabilities. This study failed to conform to any of the seven criteria of quality shown in Table 2 [no field observations, unrealistic laboratory incubation conditions, altered geosorbents, soil sterilized by autoclaving, the test compound (naphthalene) had a co-solvent (acetone), and two pure bacterial cultures were used]. Nevertheless, diversity and malleability of the microbial world became apparent when one culture was found to metabolize only aqueous-phase naphthalene; while the other metabolized a significant portion of the sorbed chemical.

In another laboratory study to determine the fate of PAHs in soil, Guthrie and Pfaender (1998; entry #12 of Table 4) used the four-ring compound, pyrene, to assess how microbial activity influenced the conversion of ^{14}C-labeled pyrene that was added to several operationally established humic acid fractions of soil. Viable soil microorganisms were found to enhance incorporation of the ^{14}C-labeled pyrene into humic acid fractions. However, aging the pyrene for 270 days did not significantly diminish its susceptibility to mineralization by soil microorganisms. The work by Guthrie and Pfaender (1998) was not conducted in the field and used several approaches that failed to conform with the criteria in Table 2.

Khan and Behki (1990) and Johnson *et al.* (1999) rigorously tackled the issue of incorporating soil-applied pesticides into humic substances and their potential subsequent release (entries #13 and #14, respectively, in Table 4). Khan and Behki (1990) formed bound ^{14}C-atrazine residues in an agricultural soil by incubating the herbicide for 1 year and then used ethanol extraction to ensure all unbound forms of atrazine were removed. Then Khan and Behki (1990) added microbial cultures to the soil and used chemical analyses to follow microbial metabolism and extractability of the soil-bound residue during an 84-day incubation. Atrazine and atrazine metabolites (indicative of microbial metabolism) were released in ppm amounts. Johnson *et al.* (1999) essentially repeated the work of Khan and Behki (1990) with methodological modifications that included aseptic preparation of bound residues (in γ-irradiated soil) during 3 months of aging. Data from Johnson *et al.* (1999) showed that bound residues were extractable and desorbable from soil but viable microorganisms did not enhance extractability. Thus, the studies by Khan and Behki (1990) and Johnson *et al.* (1999) displayed both consistency and inconsistency: bound residues were released in both studies, but the role of microorganisms in these releases was not reproduced. Neither of the studies conformed to the quality criteria in Table 2.

Entry #15 in Table 4 (Ogram *et al.*, 1985) is an often-cited report that used a combination of mathematical modeling and careful manipulation of soils and pure bacterial cultures to test the hypothesis that sorption of 2,4-D to soil protects the herbicide from biodegradation. Ogram *et al.* used sterile soil (by autoclaving), a pure bacterial culture, a co-solvent for the 2,4-D, and a brief (5 hours) incubation time for the experiments. Clearly, these experimental conditions failed to conform with the "field relevant" criteria of Table 2. It is noteworthy, however, that on numerous occasions Ogram *et al.*'s (1985) paper has been cited to support arguments that sorbed substrates may be unavailable for biodegradation. However, in the original publication, the authors were very careful to advise readers not to misuse the findings. Ogram *et al.* (1985) stated that "results...for a single strain of bacteria degrading 2,4-D in a rigorously controlled environment...may not be applicable to conditions one would expect to find *in situ*, where mixed populations may be degrading the pesticide for a much longer time than the 5-hour incubation period used here."

The final two entries in Table 4 (#16, 17) examined the influence of the residence time of agricultural chemicals (DDT and related compounds) on bioavailability, assessed via earthworm uptake instead of biodegradation. Tang *et al.*'s (1995, entry #16 in Table 4) objective was to calibrate chemical extraction techniques with pesticide bioavailability to earthworms in soil. These researchers were able to build an experimental design around soils to which DDT had been applied 30 and 49 years prior to the study. Several other combinations of pesticides and agricultural soils also were prepared in the laboratory using sterile (irradiated) soil aged in the presence of solvent-delivered, flash-evaporated pesticides. Although Tang *et al.*'s. (1999) focus was on correlating chemical extraction and earthworm uptake measurements, data presented sometimes failed to support the hypothesis that bioavailability diminishes

over time. For instance, for a Lima loam soil, aseptic aging of DDT, DDE, and DDD led to increased pesticide bioavailability to earthworms. In the study by Morrison *et al*. (2000; final entry of Table 4), chemical extraction and earthworm uptake procedures were again applied to aged and unaged pesticides in soils. In several instances, inconsistent and conflicting data indicated that long residence times of insecticides in soils did not lead to decreases in earthworm uptake from soil. Thus, Tang *et al*. (1999) and Morrison *et al*. (2000) provide examples of reports that are of secondary significance for this report. Although some of the data were obtained from field-contaminated soil, irradiated soils, flash-evaporated pesticide delivery, and inconsistent and conflicting data (see Table 2) detracted from their results.

8.3.1. Summary

Of the hundreds of research articles published in the last two decades on bioavailability and biodegradation, details of only 17 were presented in Section 8.3 and Table 4. These were chosen because they were influential and/or recent and representative of the varying approaches, objectives, and the quality of experimental designs and methodologies published to date. Each of the studies described in Table 4 had its own merits—otherwise publication would not have occurred. Furthermore, although none of the studies was designed to specifically address the needs of this report, roughly the first half of the entries in Table 4 conformed reasonably well with this report's quality criteria (field realism, environmental relevance, absence of artifacts, consistency) set forth in Table 2.

Steinberg *et al*. (1987) showed that field-aged and freshly added EDB pools in soil were chemically and biologically distinctive, and that pulverization enhanced desorption of aged EDB; thus, micropore entrapment was indicated as a cause of reduced EDB bioavailability. Studies by Olivera *et al*. (1998) and Berg *et al*. (1998) showed that petroleum hydrocarbon residues were persistent after land farming biotreatment had reduced leachability and toxicity and that additional biodegradation was likely prevented by reduced hydrocarbon bioavailability; thus, "environmentally acceptable endpoints" had been achieved by bioremediation treatment. Although laboratory-based, the study by Salanitro *et al*. (1997) also demonstrated that biotreatment of freshly added petroleum hydrocarbons in soil reduced the mobility and toxicity, hence the bioavailability, of residual contaminants. Cornelissen *et al's*. (1998) investigation showed that a portion of the rapidly desorbing (i.e., bioavailable) PAH fraction from harbor sediments corresponded to the readily biodegradable fraction. The combined field and laboratory study by Madsen *et al*. (1996) narrowed the cause of naphthalene persistence to be limitations in either bioavailability or oxygen. Scribner *et al*'s. (1992) investigation [much like that of Steinberg *et al*. (1987)] showed that the chemical and biological properties of freshly added and aged simazine were distinctive, and an aging-related reduction in simazine bioavailability was the cause. **Thus, the above investigations strongly sup-**

port the hypothesis that chemical assays of materials in soil can overestimate what is actually biologically absorbed and metabolized. Several bioassays (biodegradation, uptake, and toxicity) and chemical extraction assays indicated that the reduction in contaminant bioavailability in soil was a time-dependent phenomenon (freshly added contaminants were distinctive from aged contaminants). Such observations conform with the various operational definitions for bioavailability limitations described in Sections 3.1 and 3.3 of this report.

The entries that appeared in the latter half of Table 4 largely failed to conform with the quality criteria of Table 2. Nevertheless, each study contributed to broad scholarly information about the potential influences of geosorbents, time, reactions, and biota on biodegradation and pollutant behavior and/or toxicity in soil.

8.4 Influence of Bioavailability on Phytoremediation of Metal-Contaminated Soils

One key characteristic of plant-based, cleanup technologies is that plants can have major physical and hydrologic impacts on contaminated sites (Ensley, 2000; Schnoor, 2000). Not only do growing plant roots explore and penetrate the three-dimensional volume of contaminated soil, but the plant vascular system withdraws water from the soil depths and delivers it to the atmosphere. Transpirational demand at leaf surfaces is the driving force for the flow of water from soil solution to the root surface and into and through the plant biomass. As discussed in Section 7.2 of this report, the relative contribution of mass flow (versus diffusion versus root interception) to nutrient uptake varies with each plant species. Nonetheless, because all plants transpire, they all establish moisture gradients within the soil matrix, induce advective mass transfer of water, and convey solutes both through soil and into their tissues (Schnoor, 2000). If solutes, including metals, are in soil solution, they assuredly will be conveyed to the plant (see Section 7.1). It is for this reason that substantial research efforts have been directed at boosting access—boosting bioavailability—of metals to plants.

Mobilization of metals from soil into soil solution and plants is particularly amenable to experimentation because organic chelating agents such as ethylenediaminetetraacetic acid (EDTA) have been used for decades for similar purposes. Phytochelators (Khan *et al.*, 2000) also have been described. Many greenhouse experiments have demonstrated the effectiveness of added chelating agents in enhancing potential phytoremediation (Blaylock *et al.*, 1997). For example, when EDTA and HEDTA were added to pot-grown *Helianthus annuus*, uptake and translocation of Cd and Ni from contaminated soil was substantially increased (Chen and Cutright, 2001). Also, Ebbs and Kochian (1998) demonstrated that an EDTA amendment increased Zn accumulation by Indian mustard planted in metal-contaminated soil.

Although entry of metals into soil solution is an important aspect of phytoremediation strategies, defining soil and plant factors that govern metal uptake also is a major area of current research (McGrath *et al*., 2001). In this regard, "nonbioavailable" forms of metal have begun to be identified. For example, Stanhope *et al*. (2000) used isotope dilution techniques in pot studies of Indian mustard to show that, despite general mobilization of Cd, Zn, Pb, Ni, and Cu from sludge-contaminated soil, EDTA failed to facilitate plant access to a nonlabile pool of soil Cd. In related studies, researchers have begun to examine the time-dependence of extractability (Martinez and McBride, 20001), animal toxicity (Lock and Janssen, 2001), and plant uptake (Pedersen *et al*., 20001; Stacey *et al*., 2001) of metal contaminants. Results of such studies appear to be delivering a message much like that from microbial biodegradation studies of organic soil contaminants (see Sections 8.1 through 8.3 of this report): the relationships between metals, soils, and plants seem to vary substantially depending on the type, composition, and design of the experimental system.

8.5 A Synthesis: Evaluating the Relationships Between Bioavailability and Bioremediation Based on Sections 2 to 8.4 of This Report

This section presents a series of statements that blend lessons from the mechanistic studies, discussed in Sections 8.1 to 8.4 of this report, with the fundamentals of biodegradation, bioremediation, bioavailability, persistence of organic compounds, paradigms of geosorbent-contaminant interaction, and mechanisms of contaminant uptake by biota that were presented in Sections 2 to 7 of this report.

- **Microorganisms and plants offer a broad array of physiological and/or physical mechanisms for eliminating and/or binding organic and inorganic contaminants in soil and sediment matrices (Section 2).**
- **Soil and sediment exist in a state of kinetically constrained thermodynamic disequilibrium in which combinations of biochemical and chemical reactions, as well as gas-, liquid-, and solid-phase transport processes, cause the biogeochemical cycling of both organic and inorganic compounds (Section 6).**
- **Naturally occurring, plant-derived organic compounds constantly cycle through the soil habitat—partially entering a pool of persistent humic substances thought to be protected from microbial attack by a combination of complex molecular structure, resistance to enzymatic digestion, insolubility, failure to enter the microbial cell, possible enzyme inactivation, and complexation (masking) (Section 5).**
- **Although the details of how and why chemicals persist in soil are governed by each chemical's molecular structure, a survey of soil bioremediation field projects (for petroleum hydrocarbons, pentachlorophenol, PAHs, metals; Sec-**

tion 4) indicates that these organic and inorganic contaminants also routinely persist in soil, though simple mixing and aeration frequently accelerate organic contaminant loss.

- **Six specific mechanisms have been hypothesized as the "cause" of diminished bioavailability, hence, chemical waste persistence in soils: (1) sorption to surfaces, (2) partitioning into NAPL phases, (3) micropore- and nanopore-separation, (4) complexation, (5) insolubility, and (6) partitioning into organic matter (Section 3).**

- **Bioavailability can seldom be measured directly. Instead, it emerges from the specific, detailed, three-way interaction between biota, chemical waste compounds, and a geochemical matrix under study (Section 3).**

- **Soil biota (microorganisms and plants) feature a diverse array of anatomical and physiological adaptations (e.g., solid-phase uptake, diffusion-accelerated uptake, active transport, extracellular enzymatic digestion, extracellular scavenging systems) that allow them to successfully contend with the adverse nutritional conditions of soil (Section 7). Such physiological and evolutionary adaptations should not be underestimated when interpreting data or making predictions related to the availability of chemical wastes for bioremediation in soil. Many metabolic capabilities of soil biota (especially microorganisms) probably have yet to be discovered (Section 6.3).**

- **All six hypothesized "mechanisms of diminished bioavailability" (Section 3) involve a spatial separation of the chemical substrate from intra- or extracellular metabolism. To some degree, direct physical contact between cells (or roots) and the metabolized chemical can counteract spatial separation. However, even excretion of extracellular enzymes cannot counteract the size differential between molecules that could diffuse into soil nano- or micropores (Ball and Roberts, 1991; Newman and Thomasson, 1979) and microbial cells (≈1μm). Thus, regardless of the stage of sophistication of mathematical and conceptual models of geosorbent matrices (Section 6.4), there is no doubt that some portions of chemical waste in soil can be—and are—inaccessible to plants and microorganisms.**

- **Inaccessibility of chemical wastes in soil, synonymous with diminished bioavailability, is observed as discrepancies between modest or undetectable bioassay responses (uptake, toxicity, or biodegradation) to chemicals, compared to ready analytical quantification of the chemicals in the same soil samples (Section 8.3).**

- **The prevalence of ambiguous, even conflicting, findings in the existing literature on biodegradation and bioavailability (Sections 8.1 to 8.4) is probably a reflection of: (1) the simultaneous variability in the methods, test chemicals, approaches, and experimental systems that have been devised by numerous**

concerned investigators, and (2) the concomitant shifts in prevailing mechanisms (e.g., direct contact, extracellular enzymes, diffusion, active uptake) by which the biotic components in these investigations access and metabolize the tested chemicals.

Section 9

Overcoming Constraints on Site Cleanup

The propensity for contaminated soil to remain contaminated poses a major challenge for the proper management of contaminated sites, especially site cleanup technologies (National Research Council, 1997). Several recent publications on site cleanup techniques, particularly bioremediation measures, have reviewed current knowledge on efforts to overcome constraints on contaminant removal (e.g., Alexander, 1999; Maier, 2000; Volkering *et al*., 1997; Scow and Johnson, 1997; West and Harwell, 1992). According to Maier (2000), surfactants (biosurfactants and synthetic surfactants), cosolvents, and thermal treatment can aid in the physicochemical removal of organic contaminants from porous media.

All such contaminant-mobilizing strategies have advantages and disadvantages, and none have yet been found to be fully satisfactory. The reasons for this absence of completely satisfactory performance should be no surprise to the reader of this report. Soils and sediments are highly complex geochemical matrices and, given the variable properties of both contaminants and geochemical matrices, the impact of geosorbent treatment techniques is not likely to be consistent. The task of mobilizing chemical waste contaminants from geosorbents might be pursued for "pump and treat" purposes (simply to wash the matrix) or for bioremediation purposes (increasing contaminant availability for biodegradation reactions). Regardless of the goal, understanding the science of mobilizing contaminants out of soils and sediments is analogous to understanding the science of soil bioremediation (e.g., Figure 1; Section 3.3). Thermodynamically governed, complex interactions between the geosorbent matrix and the contaminants cannot be escaped. If Figure 1 was to be adapted to address mobilization of contaminants from soils, the label on the oval in Figure 1 should be replaced by "contaminant mobilization treatment." Then the performance of the system in the revised Figure 1 would be assessed, not on bioremediation, but simply soil washing efficacy. The "contaminant mobilization treatments" examined to date have often been plagued with implementation difficulties that range from high cost to inefficiency, secondary chemical effects, and

secondary biological effects (toxicity, degradation of water quality, inhibition of biodegrading microorganisms).

Because potential soil amendments feature a diversity of chemical structures and their corresponding properties, there are still many promising approaches for geosorbent decontamination efforts to be devised and tested. Noordman *et al*. (1998) have explained that aquifers contaminated with hydrophobic organic contaminants can theoretically be remediated using dissolved organic matter, cyclodextrins (e.g., McCray and Brusseau, 1999), organic cosolvents, or surfactants (Guha *et al*., 1998). Rhamnolipid biosurfactants may be superior to the alternatives because they are naturally occurring compounds with low environmental impact (Noordman *et al.*, 1998; Torrens *et al*., 1998). Surfactant treatment of soils and sediments occurs via emulsification, micellar solubilization, or facilitated transport mechanisms. Volkering *et al*. (1997) reviewed many efforts to link surfactant-mediated mobilization of contaminants to bioremediation: results ranged from stimulation to inhibition of both desorption and biodegradation of polluting compounds. Volkering *et al*. (1997) concluded that "no general trends can be found... Therefore, more research is necessary to make the application of surfactants a standard tool in biological soil remediation."

Regarding phytoremediation of metal-contaminated soil (see Section 8.4 of this report), there is no doubt that when chelating agents boost the concentration of metals in soil solution, metal uptake by plants will be enhanced. However, subtle secondary effects of such amendments on soils, plants, and real-world field sites have yet to be fully elucidated.

Section 10

Conclusions, Implications, and Possible Areas of Future Research

This section focuses all prior portions of this report upon key facts, principles, and their implications.

Three Scientific Conclusions:

1. Based on a conservative, reasonably thorough and careful evaluation of scientific studies described in this report, there is no doubt that chemical wastes in soil can be, and often are, in a state of reduced bioavailability.

2. Reduced bioavailability simply means that a chemical waste's diminished "effective concentration" is proportionately balanced by a lingering reservoir of the chemical waste in soil and sediments. This lingering reservoir remains in the soil habitat regardless of which combinations of conceptual or actual sequestration mechanisms (e.g., complexation into bound residues, diffusion into soil pores, NAPL partitioning) apply.

3. Soil is, by definition, a thermodynamically unstable, kinetically constrained medium whose chemical composition, including solid, liquid, and gaseous components, is constantly changing. Thus, the "nonbioavailable" chemical wastes in this lingering reservoir (point #2) are always subject to release into soil solution where the wastes are resubjected to a variety of transport and/or transformation processes (e.g., immobilization, biodegradation, uptake by receptors).

Implications of Bioavailability:

1. Considerable effort has been expended in investigating the hypothesis that chemical wastes have diminished bioavailability in soils and sediments. Results of these efforts have been ambiguous because of the immense diversity in types and properties of chemical wastes, geosorbents, biota, experimental approaches, and the idiosyncrasies in mechanisms by which biota interact with chemical wastes.

2. From a practical, regulatory point of view, establishing the foundation of reduced bioavailability is crucial. If the reduced bioavailability of chemical wastes in soil becomes widely accepted, then proper quantitative measures of bioavailability reduction could be developed to accurately estimate the risks posed to human health and ecological processes by chemical wastes in soils and sediments.

3. An accurate estimation of risks to human and environmental health posed by chemical wastes in soils (point #2) is, itself, a crucial step toward: (i) identifying pragmatic, economically feasible environmental cleanup goals; (ii) establishing operational definitions of "treatment" by bioremediation technology; (iii) realistically classifying polluted sites based on planned land-use scenarios; (iv) developing public acceptance of risk-based contaminant cleanup efforts; (v) developing public acceptance of cleanup goals that are above the "original, pristine state" of the contaminated site, and (vi) legitimizing the concept of "environmentally acceptable endpoints."

Possible Areas of Future Research:

1. Microbe- and plant-based bioremediation technologies are not able to remove 100 percent of contaminants from polluted sites. Thus, managing residual contaminants in real-world sites will require progress in the science of contaminant behavior, in risk assessment, and in site management polices.

2. Several major questions remain about the reservoirs of "nonbioavailable" chemical wastes in soil:

   A. Is it possible to determine the rates of soil processes in the field that convert "nonbioavailable" chemical wastes to available forms?

   B. What methods are appropriate?

   C. Can these appropriate methods be applied in regionally representative field sites containing representative classes of organic and inorganic chemical wastes?

   D. Can the results of item #2C be used to formulate region-specific, site management guidelines?

   E. When released into soil solution, what are the relative probabilities and rates of the chemical wastes becoming:

      – resequestered?

      – biodegraded?

      – transported to susceptible receptors?

   F. If the released chemical wastes are readily mineralized (if organic) or immobilized (if inorganic), would "natural attenuation" of these originally

nonbioavailable contaminants offer effective protection of human health and the environment?

3. Several major questions remain about the true, field state of solid, liquid, amorphous, and metastable organic and inorganic constituents in soils and sediments:

   A. What are the chemical forms and locations of soil constituents?

   B. What types of surface chemical and/or other reactions predominate?

   C. What effects do these reactions have on sequestration and/or release of chemical wastes?

   D. At what rates (minutes, days, decades, centuries) do these reactions occur?

4. The types of basic scientific questions raised in item #2 also apply to contaminants and their reactions in soils and sediments.

5. The questions raised in item #2 apply to soil biota—especially to the soil microbial community—because so much of its diversity and potential physiological activities are uncharacterized.

6. Would modeling of the processes in items #2-5 provide predictive, heuristic tools to regulators, the public, and scientists?

7. Can the risks of release of initially "nonbioavailable" soil contaminants to soil solution be estimated in a meaningful way that accounts for:

   A. Critical pathways at each contaminated site that could lead to exposure to humans or other key receptors?

   B. An integration of known mechanisms that contribute to both the re-release and re-sequestration of soil contaminants?

8. Can knowledge of the mechanisms that lead to persistence of chemical wastes in soils (i.e., resistance to biodegradation and/or physical/chemical immobilization) be understood well enough to manage the mechanisms at field sites in ways that either enhance or counteract sequestration reactions, as appropriate for site management goals (e.g., Verstraete and Devliegher, 1997)?

9. Can researchers be convinced to use methodologies and experimental designs that avoid artifact-laden experimental results and resist the temptation to publish treatment differences whose practical, long-term implications are moot in the real world?

10. Do we need to anticipate and measure potentially adverse, secondary effects of bioremediation technology? These effects could result in nutrient limitations and/or inadvertent release from soil of undesirable metals or chemical metabolites.

11. Research efforts to address bioavailability and bioremediation data have produced a broad spectrum of information about possible reactions that might occur between geosorbents, contaminant wastes, and biota. This substantial roster of possibilities needs to be narrowed to identify what actually does happen in real-world field sites. Can the true field behavior of contaminants be systematically sampled and interpreted so as to enable robust predictions of contaminant behavior based on climate, region, contaminant class, and soil type?

Section 11

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