

Sequestration and Bioavailability of Organopollutants in Soil: Their Implication to the Risk Assessment

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ABSTRACT : The bioremediation is an economic technology to remove the organopollutants from soil. It is often found that the remediation could not remove the compounds below the levels determined by vigorous extractions as required by regulatory agencies. The reason for the reduced bioavailability with increasing time of aging has been accredited to the sequestration of the compounds in remote sites within or between soil particles. Then, the aging could be defined as the time-dependent sequestration. Partitioning and entrapment have been suggested as mechanism for aging. The rate and extent of the sequestration varies among dissimilar soils. The bioavailability of aged pollutants in soil could be measured by bioassays, mild solvent extraction, and soil-phase extractions. The sequestration could be affected by many factors including various soil properties, wetting and drying cycle, and the presence of cosolutes and NAPLs etc. The bioavailability and sequestration should be considered to determine the environmentally acceptable endpoint.

Key Words : Extractability, Aging, Risk assessment, Sorption, Desorption

Introduction

Many field soils have been contaminated with various organopollutants long before bioremediation is considered to clean up the sites. In some cases, the polluted sites have not been concerned and abandoned over several decades before strict environmental laws come into actions. By the time the polluted fields were remediated, it has been found that some portion of chemicals in soil are very resistant to the biodegradation by microorganisms. That might be because the pollutants have been in contact with the soils for an extended period of time, become weathered, and been complexed with soil components (Alexander, 1999; Alexander, 1995). In recent years, evidence has accumulated that certain slow processes reduce the availability and thus the toxicity of the compounds in soils with time. The slow processes termed aging are supported by a host of data indicating that organic molecules are slowly sequestered within soil matrix. The aging, as marked with the declining bioavailability and sequestration, may affect the potential risk posed by the pollutants, the appropriate remediation technologies, and the establishment of regulations for the cleanup of sites containing hazardous chemicals (Alexander, 1999, 1997, 1995; Loehr and Webster, 1996).

Several lines of evidence point to the sequestration of the chemicals that persist in soil. Many recent studies showed that increased contact time between chemicals and soil decreased the availability of the chemicals toward the microbes responsible for biodegradation, making them more difficult to be bioremediated. In addition, the increased contact time also decreased the extractibility of the chemicals from the soil. Sorption and desorption may be important fate mechanisms for chemicals in soil (Alexander, 1999, 1997, 1995; Loehr and Webster, 1996). Alexander (1999) summarizes hocky-stick pattern of sorption and desorption mechanisms where each one follows a two-phase pattern, a rapid phase followed by a much slower phase. The toxicity of many pesticides to plants and insects declined with increasing residence time in soil (Alexander, 1999). All the evidences mentioned above indicate that there are underlining scientific basis for the sequestration and bioavailability of organic chemicals in soil. The basis may be critical for us to determine an environmentally acceptable endpoint and develop an economic remediation technology.

Sorption and Desorption of Organic Compounds

Many chemicals are biodegradable but are not biodegraded in some situations (Alexander, 1999). There could be many reasons. One of them may be due to the sorption of the chemicals to the solid surfaces. Thus, the chemicals are not easily available for the microorganisms. The surfaces can be in many forms such as clay minerals, organic matter, or amorphous metal oxides in soil. The solid surfaces in soil can retain solute on the surfaces (adsorption). However, in some cases, the solute may be retained within the mass of the solid rather than on its surfaces (absorption). To understand the sorption process, we need to know both the kinds of chemicals and the physicochemical properties of solids. Specific environments around which the sorption occurs, such as pH and temperature etc., may be of great importance to the sorption. Chemicals may be negatively or positively charged. However, many of the organopollutants are hydrophobic. Thus, our attention can be focused on the behavior of the hydrophobic compounds in soil. Much attention has been given to the sorption of organic compounds both to the clay and organic matter (Chiou, 1989). Clay may have either nonexpanding or expanding minerals. While nonexpanding clays such as kaolinite are assembled in a 1:1 ratio of Si and Al, expanding clays such as montmorillonite have a layer in a 2:1 ratio (Dixon and Weed, 1989). The distribution of clay type has a big deal of influence for the sorption and desorption of the organic compounds since it determines the availability of both outer and internal surfaces of clay minerals. Especially, the expanding clays frequently sorb chemicals in the expandable lattice structure where the organic compounds can be protected from the microbial attack (Alexander, 1999). The organic fraction of soils is responsible for the sorption of many hydrophobic compounds. For example, many polycyclic aromatic compounds are more strongly sorbed by organic matter in soil than by clay mineral. Thus, the extent of this retention is directly correlated with octanol-water partition coefficients (K_{ow}) and the content of organic matter in soil. The organic matter can sorb the chemical in two possible modes. In the first mode, the solute binds physically to the organic solid thus the molecule is concentrated on the outer surface or within the pores of a solid (Calvet, 1989). In another mode, the solute diffuses into the organic matter and is contained within organic matter (Chiou, 1989). In either cases, the bioavailability of sorbed organic compounds will depend on the specific sorption mechanisms as well as the strength of bonding involved in the retention.

Evidences exist showing that increased contact time

between chemicals and soil leads to stronger sorption and greater resistance to desorption. Robinson et al. (1990) conducted experiments to determine the sorption characteristics of toluene added to soil: the relationship between concentrations of chemicals sorbed to soil (C_s) and that in aqueous solution (C_w). The results showed that C_w had dropped significantly in a week but the dropping became slowed down with increasing time (4 and 14 weeks). This indicated the two phase sorption process: an initial rapid sorption period was followed by continuing adsorption at progressively slower rate. They also investigated the desorption characteristics of toluene in soil. Toluene was added to a soil and 13 sequential water extractions were done for 2 days. Most of the sorbed toluene was extracted with 4 extractions and 90% of the toluene was recovered over 13 extractions. However, about 10% of the toluene could not be extracted through the water extraction or a final methylene chloride extraction. More experiments revealed that this resistant fraction did desorb only at very slow rates. This also showed that the overall toluene desorption occurred in two stages as we have seen above with the toluene sorption experiment. Pavlostathis and Mathavan (1992) have added trichloroethylene (TCE) to several soils to study the effects of contact time on desorption. The TCE was allowed to equilibrate with the soil for various periods. The extent of desorption with TCE decreased from 90 to 65% with the increase in contact time from 2.5 to 15.5 months. Desorption partition coefficients increased with increased contact time. The partition coefficients have been correlated with the fraction of chemical that resisted desorption. Many other studies showed a similar phenomenon with phenanthrene in sandy sediment (Fu et al, 1994), naphthalene in field soil (Connaughton et al., 1993), ten halogenated alkanes and alkenes in two surface soils where they formed slowly reversible sorbed fractions (Pignatello, 1990).

The residual fraction was correlated with the soil organic matter. The size of the residual fraction generally increased with increasing organic matter content, although nonlinearly. Karickhoff et al. (1979) found that the partition coefficients of hydrophobic chemicals were directly related to soil organic carbon content. Thus, the effect of contact time on sorption and desorption may be more pronounced in soils with high organic carbon content. Manilal and Alexander (1991) found that the rate of phenanthrene mineralization was inversely correlated with the organic matter content, suggesting that sorption by soil organic matter slows the biodegradation of the

hydrocarbon in soil. Many studies showed that the inhibition of bioavailability of chemicals is due to the sorption of the chemicals: polycyclic aromatic hydrocarbons (PAHs) (Weissenfels et al, 1992) and EDB (Steinberg et al., 1987).

It has been once believed that the rate of microbial degradation of sorbed compounds by microorganism is limited by the rate of desorption (Bedard, 1990). However, although sorption often reduces the rate and extent of biodegradation, it may not be necessarily prevent it. Manilal and Alexander (1991) have shown that when all chemicals are sorbed biodegradation occurs. The availability of sorbed hydrophobic compounds is evident in a study of phenanthrene sorbed to polyacrylic beads (Tang et al., 1998b). They showed that the rate of degradation of the sorbed PAH by bacterium P5-2 was faster than the rate of its desorption determined in the absence of microorganisms, while bacterium P3 could not mineralize the hydrocarbon sorbed to the hydrophobic surface. Some other studies also showed the faster biodegradation with decylamine (Wszolek and Alexander, 1979) and biphenyl (Calvillo and Alexander, 1996). The utilization of a compound at a rate faster than its abiotic desorption requires the existence of a mechanism that facilitates degradation of sorbed chemicals: the production of biosurfactants (Scheibenbogen et al., 1994) or the direct attachment of microbes to the surfaces (Calvillo and Alexander, 1996).

Sequestration and Aging: Definition and Examples

When an biodegradable compound is introduced into soil, the compound have several fates. Some are washed out by rainfalls or lost by volatilization. Some are in solution or on the surface that is easily accessible by indigenous microorganisms to be degraded. Soil has a complex structure which is composed of particles and pores of various sizes. A chemical may diffuse into a micropore or move out of it to a site where microorganisms can be habitable. Others diffuse into the remote pores in soil and are not accessible by the microbes. Another possibility is that some of the compound are complexed (or bound) with soil organic matter (i.e., humin, fulvic acid, humic acid) and thus not easily available for microbial destruction (Singh and Agarwal, 1992). The compound that remains is often termed an aged chemical. However, the term aging may mean that the chemical is modified rather than intact. Because some of the compound is still intact and become inaccessible, Alexander (1995)

introduced the term sequestration be distinguished from the compound complexed with soil components. Also the term sequestration refers to a loss in availability of a compound, and the term aging refers to the time required. The sequestration and aging process is quite related with the tortuosity by which a compound find a difficulty in reaching the place where microorganisms exist. Considering the dimensions of soil microbes ($\sim 2 \mu\text{m}$), there are a large portion of clays and humic surfaces are free of microorganisms. Hassink et al. (1993) reported that pores with diameter $< 0.2 \mu\text{m}$ occupy 30% of the total volume of some soils. Additionally, the accessibility of microbes would be even further occluded by the ink-bottle necks that impede the movement of even mobile cells that otherwise easily move the other side of the neck (Alexander, 1999).

The results of monitoring of the disappearance of a large number of biodegradable compounds in soil suggest that they undergo a time-dependent decline in bioavailability (Alexander, 1999, 1997, 1995; Loehr and Webster, 1996). Because of its time-dependance, it may be appropriately called aging and somewhat different from that involved in rendering the compounds less available. Sequestration of chemicals during aging process is characterized by hockey stick-shaped kinetics (Alexander, 1999, 1995). In general, the compound disappears rapidly in the initial stage and the disappearance slows down with increasing residence, and finally its disappearance almost stops. However, the extent of the remaining compound (that is, not biodegraded) as well as the rate of disappearance of the compound would be different with dissimilar soils. This phenomenon has been observed with three field soils contaminated with DDT, where the initial concentrations of DDT were 200mg/kg, 4.42mg/kg, and 11.2kg/ha and the time scale was about 2.5, 4, and 17 years, respectively (Alexander, 1999). Many insecticides have shown a similar hockey stick pattern (Alexander, 1995). Much of the chemical reported so far can be removed from soils by vigorous extractants (without acids or bases: thus it is not complexed) but the aged compound is apparently not biodegraded. However, the freshly added compounds in the same kinds of soil are biodegraded by microbes (Loehr and Webster, 1996). The aged compounds remained in soil could be problematic if the concentration of the chemical is well above the governmental regulation.

Steinberg et al. (1987) compared the availability of 1,2-dibromoethane (EDB) aged in two agricultural top soils for 0.9 and 3 years with that freshly added EDB (spiked). They found that while $\sim 90\%$ of the freshly added EDB was degraded

in 4 and 22 days in each soil, respectively, no degradation of EDB was observed in either of the aged soils. The release of EDB into aqueous solution was studied using a gas purging technique to recover desorbed EDB from the aqueous solution. Freshly added EDB was easily removed from the soil, while EDB in soil aged for 0.9 years was removed at a much slower rate. One hundred minutes of purging removed all of the freshly added EDB and <5% of the aged EDB. Scribner et al. (1992) showed that simazine accumulated in a soil due to the 20 times application for 20 years remained the same but the concentration of the freshly added herbicide declined greatly in 7 weeks. Weissenfels et al. (1992) investigated the biodegradation of PAHs in soils obtained from two different industrial sites under simulated land-treatment conditions. PAHs in soil A were readily degraded and not that in soil B. The addition of PAHs-degrading bacteria to soil B could not enhance the biodegradation. However, when extracted PAHs from soil B were added to a clean soil or back to the soil B, PAHs-degrading inoculum degraded 72% of the total PAHs. This results indicated that aged PAHs in soil B might penetrate to a remote site so that the bacterium could not reach for biodegradation. These facts also indicated that freshly added chemicals do not accurately mimic the behavior of chemicals in soils found at a contaminated field that have been exposed with the chemicals for a long period of time.

Another evidences have been summarized at the simulation study in the laboratory where each compound is allowed in sterile soil for varying periods of time (Alexander, 1999, 1997; Loehr and Webster, 1996). At the study, any other fates of the chemicals except sequestration with time have been tried to be prevented and the use of chemicals that do not easily complex with soil components has been used. After different incubation times with soils spiked with chemicals such as phenanthrene, atrazine, 4-nitrophenol, the aged soils were subject to the mineralization by each corresponding microorganisms. All aging studies with various soils showed that the extent of biodegradation declined with increasing periods of time. When mass balances were performed on the uninoculated soils, nearly all of the compounds has been recovered. In addition, the vigorous extraction after bioremediation revealed that the amount of the compounds remained (the fraction that resisting biodegradation) increased with aging. Not only the availability to microorganism of chemicals decreased but also the extractability by mild extractants of the compounds. It should not be confused with vigorous extractants (i.e., soxhlet extraction with methylene chloride) that give quantitative

recoveries of organic compounds shortly after their introduction into soil (Alexander, 1999; Loehr and Webster, 1996). Kelsey and Alexander (1997) showed that phenanthrene and atrazine in sterile soil, with time, became progressively less extractable with mixtures of methanol and water. This diminished extractability of chemicals with time has been observed with many other studies, indicating that a change is occurring that renders persistent compounds less accessible, whether accessibility is measured with biological or chemical methods (Alexander, 1999; Loehr and Webster, 1996).

The decline in availability has been observed in other species other than microorganisms. In an old study, the toxicity of lindane to *D. melangogaster* (fruit flies) in a loamy soil declined to a far greater extent after 22 months than did the concentration of the chemical as determined by solvent extraction (Edward et al., 1957). Peterson et al. (1971) found that the toxicity of DDT to the fruit flies was much reduced in 108 days period. Similar results have been reported with plants. Bowmer (1991) showed that much of the aged atrazine in soil is not toxic to plants. In more recent study by Tang (1998a), uptake of anthracene by wheat and barley decreased with increasing time of aging. Robertson and Alexander (1998) presented another evidence that acute toxicity of two pesticides in sterile soil declined greatly with aging. The lethality of dieldrin decreased from 100% to none in 120 days in tests with fruit flies and cockroaches, and the lethality of DDT and dieldrin also decreased to zero in assays with house flies. However, the quantities of the pesticides that were extracted with vigorous extractants was more than 84% of the initially added concentrations. This results also suggested that the analytical values obtained by the vigorous chemical extraction did not correlate with that by biological methods. Tang et al. (1998a) obtained a similar result with earthworm that the amount assimilated of various PAHs by the animals in sterile soil diminished with increasing time of aging.

Factors Involved in Sequestration and Aging

We have seen many evidences about sequestration and aging with which the availability of organic compounds in soil diminishes with time, as measured by various biological and/or chemical measures. There are two hypotheses to explain the sequestration and resultant decline in bioavailability of organic compounds. Hydrophobic organic compounds sorb rapidly to external surfaces of soil and then slowly partition into interior regions of the soil organic matter (SOM). Thus, the

partitioning of the compounds into SOM has been suggested as a mechanism for the sequestration. Xing and Pignatello (1997) have suggested that SOM is a dual mode sorbent having both rubbery and glassy characteristics so that sorption occurs by both a partition mechanism and a hole filling mechanism. Regardless of its action mechanism, SOM should be one of the major determinant. Steinberg (1987) suggested entrapment of the compounds in micropores as another mechanism for the sequestration. Thus, the compounds are proposed to diffuse into micropores within the aggregate structure or nanopores and, with time, enter increasingly remote sites that are not accessible by even the smallest bacterium. Nanopores are surprisingly common in soils. Alexander (1999) have shown that the distribution of pore in size between 10 nm to 10 μ m is very common in field soil and varies greatly in different soils. The existence of the minute pores also means that the total surface area due to the nanopores is enormous. In addition, Mayer (1994) proposed that organic matter in marine sediments was protected by its location inside pores that is too small to allow for the entrance or functioning of hydrolytic enzymes. Now we can envision better about sequestration and aging. Organic compounds introduced into soil are slowly diffuse into nanopores while they repeatedly sorb to and desorb from soil components such as organic matter. The more time is allowed the more the compounds move into remote sites between particles or within particles. In the SOM, the rate of diffusion is orders of magnitude slower than in water (Brusseau et al., 1991). Thus, once sequestered (or aged) in the remote sites, the organic compounds would move very slowly through the three dimensional and tortuous maze to the outer surfaces where they could be bioavailable. Considering all the findings above, we can define aging as time-dependent sequestration that organic molecules make a continuous and slow diffusion into more remote sites where the compounds are retained.

White et al. (1997) investigated factors affecting sequestration and bioavailability of phenanthrene in several soils. However, no specific effect was observed with soil aggregates, clay content, and organic matter content, possibly due to the complexity and heterogeneity of the soils. Nam et al. (1998) suggested that the organic matter content of soil is a major determinant of sequestration. Later, Chung and Alexander (1998) have studied the effect of dissimilar soils on the sequestration. They found that atrazine and phenanthrene became sequestered in each of the soil but the rate and extent of sequestration varied markedly among the soils. In addition,

the extent of sequestration of the two compounds was in the 16 soils was not highly correlated and the decline in bioavailability in the soils were not highly correlated with the decreases in extractibility. However, a recent study showed a possibility that the extent of sequestration of an organic compound could be predicted by physicochemical properties such as nanoporosity, organic matter content, clay content, surface area and so forth (Chung and Alexander, unpublished data). In using the properties for the prediction one should exert caution that various soil properties could be correlated. Chung and Alexander (1999b) have reported that the most of the larger nanopores ($10^4 - 10^2$ nm) exist in the organic fraction and most of the smaller pores ($< 10^2$ nm) are present in the clay fraction. They also mentioned that qualitative differences in organic matter and clay types should be considered. The concentration of a compound often affects its behavior in soil. For example, the frequent existence of nonlinear sorption isotherms for organic compounds introduced into soil suggests the saturation of sorption sites (McBride, 1994). Such a saturation would be reflected in a declining percentage of the chemical that is sequestered as the concentration increases. In agreement with the expectation, Chung and Alexander (1999a) reported that, although the sequestration of phenanthrene and pyrene occurred at both low and high concentrations, the amount sequestered increased but the percentage decreased with increasing concentrations.

In environments as physically and chemically complex as soils, it is difficult to clearly define the mechanism of processes that account for a time-dependent diminution in bioavailability. Hatzinger and Alexander (1997) designed an experiment where synthetic polymers, waxes, and alkanes were selected as models for the organic solids and silica particles for the nanopore network. The results showed that the degree of protection from biodegradation was dependent on characteristics of the solids chosen and the rates and extents of phenanthrene mineralization declined as the percentage of the substrate in the nanopores with in silica particles, although not significant. Nam and Alexander (1998) have employed another model systems to test what factors are responsible for sequestration and aging. The results reported that the rates of biodegradation in the presence of beads having no hydrophobic surfaces were essentially the same as particle in solution. But little biodegradation occurred when phenanthrene was sorbed to hydrophobic beads made of polystyrene that contained 5 or 300 - 400 nm pores, and the sorption of the compound to the bead was rapid and the desorption very slow. This suggested that

the bioavailability of a hydrophobic compound can be markedly reduced by particles bearing nanopores having hydrophobic surfaces.

Application to the Real World: Risk Assessment

Most polluted soils were contaminated at least many years ago. Thus, sequestration is particularly significant, considering its relation to bioremediation and the toxicology of poorly degradable chemicals. Although many studies have revealed factors affecting the sequestration and aging, there are more things that we do not understand well. However, we can use known facts to enhance the bioavailability of organic compounds in soil to meet the level permitted by regulatory agencies or to reduce the toxicity of the compounds in soil. Slurrying soil in a bioreactor increased ten times the rates of degradation of lubricating oil than in plots of soil (Rittman and Johnson, 1989). More recent study by White et al. (1999) showed that slurrying soil containing unaged or aged phenanthrene and di(2-ethylhexyl)phthalate (DEHP) dramatically enhanced the rate and extent of biodegradation. The reason for the stimulatory effect of slurrying could be that the agitation improves dispersion of the compounds and bacteria and disrupts aggregates etc. It may be reasonable guess that the physical disruption increases the rate and extent of aged organic compounds in soil since new surfaces may appear. Pulverization of the soil containing EDB for 0.9 and 3 years resulted in accelerated release of the aged EDB into water and the vapor phase (Steinberg et al., 1987). It is likely that mass transfer of the chemical was enhanced by the mechanical breakup. Hatzinger and Alexander (1995) have observed that the sonication of soil aggregates containing unaged and aged phenanthrene increased the rate of mineralization, while the sonication had not effect on the extent of mineralization.

White et al. (1999) showed that the nonionic surfactant significantly enhanced the biodegradation of aged phenanthrene and DEHP in soil. Aronstein and Alexander (1992) showed that the addition of nonionic surfactants enhanced the degradation of phenanthrene and biphenyl sorbed to soil. They suggested that surfactants enhance biodegradation by solubilizing or emulsifying the sorbed compound. There are some other evidences that a sequestered compound could be made more available for biodegradation. White et al. (1998) have reported that the mineralization of sequestered DEHP was greater in

soil that was subject to wetting and drying cycles than in soil incubated at constant moisture and that the rate and extent of biodegradation of aged phenanthrene by *Pseudomonas* sp. were enhanced by the addition of anthracene or pyrene, although the organism could not degrade the two PAHs. They suggested that the enhanced bioavailability might be due to the competitive displacement.

Assessment of exposure of living organisms to toxic chemicals in soil requires information on the concentration that is available to those species. However, it has been observed that a portion of a compound that is sequestered is available to different degrees to dissimilar organisms especially if the organism has mechanisms to release the compound from the aged soil (Alexander, 1999, 1997). Then, we need to consider the cost of the evaluation to measure the degree of bioavailability using various organisms: the cost of bioassays are extremely high. An approach based on mild extraction with organic solvent in place of bioassay using earthworm was one promise (Kelsey et al, 1997). Tang et al. (1999) and Morrison et al. (2000) have developed the solid-phase extraction where they employed C18 membrane disk and Tenex TA beads, respectively. The results indicated the chemical assays were highly correlated with earthworm uptake of hydrophobic compounds. At sites of contamination where soils typically contain many compounds, the sequestration of one compound is probably affected by the presence of others. Often one or more compounds may be introduced long before others are applied to soil, and the sequence of introduction likely has an influence. Many chemicals in polluted soils are dissolved in nonaqueous-phase liquids (NAPLs), and it is not clear how the presence of such liquids or partitioning of target compounds from these nonaqueous liquids affect their sequestration.

In conclusion, all the findings mentioned above may be of great importance to establish the environmentally acceptable end-point as well as the economic bioremediation strategies. Vigorous extraction removes both the bioavailable and the sequestered fraction of the target compounds so that it overestimates the concentration accessible to living organisms. Bioremediation often does not reduce the level of target chemicals below regulatory standard based on the vigorous extraction methods. Thus, the remained amount might not pose little risk. Therefore, although the regulatory procedure suggests a failure, the result of the remediation may have met its actual objectives.

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