

Are Bound Residues a Solution for Soil Decontamination?

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Processes that cause immobilization of contaminants in soil are of great environmental importance because they may lead to a considerable reduction in the bioavailability of contaminants and they may restrict their leaching into groundwater. Previous investigations demonstrated that pollutants can be bound to soil constituents by either chemical or physical interactions. From an environmental point of view, chemical interactions are preferred, because they frequently lead to the formation of strong covalent bonds that are difficult to disrupt by microbial activity or chemical treatments. Humic substances resulting from lignin decomposition appear to be the major binding ligands involved in the incorporation of contaminants into the soil matrix through stable chemical linkages.

Chemical bonds may be formed through oxidative coupling reactions catalyzed either biologically by polyphenol oxidases and peroxidases, or abiotically by certain clays and metal oxides. These naturally occurring processes are believed to result in the detoxification of contaminants. While indigenous enzymes are usually not likely to provide satisfactory decontamination of polluted sites, amending soil with enzymes derived from specific microbial cultures or plant materials may enhance incorporation processes.

The catalytic effect of enzymes was evaluated by determining the extent of contaminants binding to humic material, and - whenever possible - by structural analyses of the resulting complexes. Previous research on xenobiotic immobilization was mostly based on the application of ^{14}C -labeled contaminants and radiocounting. Several recent studies demonstrated, however, that the evaluation of binding can be better achieved by applying ^{13}C -, ^{15}N - or ^{19}F -labeled xenobiotics in combination with ^{13}C -, ^{15}N - or ^{19}F -NMR spectroscopy. The rationale behind the NMR approach was that any binding-related modification in the initial arrangement of the labeled atoms automatically induced changes in the position of the corresponding signals in the NMR spectra. The delocalization of the signals

exhibited a high degree of specificity, indicating whether or not covalent binding had occurred and, if so, what type of covalent bond had been formed.

The results obtained confirmed the view that binding of contaminants to soil organic matter has important environmental consequences. In particular, now it is more evident than ever that as a result of binding, (a) the amount of contaminants available to interact with the biota is reduced; (b) the complexed products are less toxic than their parent compounds; and (c) groundwater pollution is reduced because of restricted contaminant mobility.

INTRODUCTION

As a result of current agricultural and industrial practices, the environment has become contaminated with excessive quantities of man-made chemicals. Many of these xenobiotics are highly resistant to natural degradation processes and tend to accumulate in soil, ground water and waste water. There is great concern over the potential toxic, carcinogenic and teratogenic nature of these compounds, and extensive efforts are now being taken towards cleaning up polluted sites. However, many of the physical-chemical methods presently available for decontamination purposes involve large-scale excavation or long-term treatment, either of which may be cost-prohibitive and/or ineffective. Consequently, there is an immediate need to develop alternative methods for the *in situ* decontamination of soil and aquatic environments.

It is well known that a large portion of the chemicals that reach the soil become sequestered or bound to it. This phenomenon was well documented when it was shown that between 20 and 70% of pesticides or their degradation products resisted extraction from soil by water and organic solvents (Calderbank, 1989). Subsequent research in the laboratory and practical experience have uncovered very little negative or toxic impact of bound xenobiotics on the environment; consequently the immobilization of pollutants in the soil has been recognized as a promising decontamination technique.

Our studies on binding mostly involved phenols and aromatic amines (Park et al., 2000; Dec and Bollag, 2001). Many of these man-made chemicals resemble naturally occurring humic acid precursors; therefore they can be incorporated into humus during the humification process. The binding prevents the compounds from interacting with the biota, and therefore reduces the toxicity of the pollutants. Binding also restricts the movement of contaminants through leaching, thus preventing the contamination of aquatic environments. The incorporation of

xenobiotics into humus is a naturally occurring process that can be enhanced through the addition of biological (enzymes) or abiotic catalysts, or by altering the physico-chemical conditions of the soil environment.

The research presented here focuses on the mechanism by which halogenated phenols and anilines as well as less reactive chemicals are bound to humic materials and on the chemical nature of the bound residues (Bollag et al., 2002). Our results indicate that enzymatic coupling provides a promising method for decontamination of polluted environments.

REACTIONS BETWEEN XENOBIOTICS AND HUMIC MATERIAL

Binding to humus constitutes one of the major reactions by which anthropogenic compounds are transformed in nature. Contaminants interact with soil colloids through several mechanisms, and a number of reviews describe the various possibilities (Pignatello, 1989; Koskinen and Harper, 1990). Adsorption occurs primarily as a consequence of the attraction between the solid surface of soil and the soluble or vapor phase of the xenobiotic. Phenolic compounds adsorb to soil colloids via several mechanisms including van der Waals forces, charge-transfer complexation, hydrogen bonding, and hydrophobic interactions (Khan, 1978). The nature and strength of adsorption depend largely on the chemical class or structure of the molecule. Adsorption is a reversible process and the released chemicals are available to interact with the biota. However, there is abundant evidence suggesting that with longer exposure to soil, adsorbed residues become more resistant to extraction and degradation (Calderbank, 1989; Hatzinger and Alexander, 1995). This resistance may result from a gradual sequestration or slow incorporation of the pollutant into humus.

The most persistent complexes result from covalent binding of xenobiotics to humic material. These complexes, often referred to as the "bound residues", are highly resistant to acid and base hydrolysis, thermal treatment, and microbial degradation (Helling and Krivonak, 1978; Katan et al., 1976; Roberts, 1984). Oxidative coupling is one of the most important chemical reactions in the humification process. It leads to the incorporation of both naturally occurring humic acid precursors and synthetic phenolic compounds into soil humus via covalent linkages. The oxidative coupling of phenols is a free radical reaction. The resonance stabilized free radicals which are formed upon the loss of an electron and a proton from phenol couple in a variety of ways. Phenolic reactants are mostly

linked through C-C and C-O bonds, while aromatic amines form C-N and N-N linkages (Sjoglad and Bollag, 1981).

Oxidative coupling is mediated by a number of biological and abiotic catalysts, including microbial enzymes, inorganic chemicals (e.g., ferric chloride, cupric hydroxide) and clay minerals (Bollag, 1983; Wang et al., 1986). Coupling reactions can also occur spontaneously in the presence of oxygen at neutral and alkaline pH. Spontaneous reactions frequently lead to the incorporation of nonphenolic compounds into humic polymers.

Many soil microorganisms produce extracellular oxidoreductases capable of catalyzing the coupling of aromatic compounds. These enzymes are classified as either peroxidases or polyphenol oxidases. All peroxidases contain an iron porphyrin ring and require the presence of peroxides (e.g., hydrogen peroxide) for activity. In particular, horseradish peroxidase (HRP) catalyzes the polymerization of a wide range of phenolic and anilinic compounds and its use in the detoxification of industrial wastewater has been examined (Klibanov et al., 1980; Maloney et al., 1986; Dec and Bollag, 1994).

The polyphenol oxidases are divided into two subclasses, laccases and tyrosinases. Both enzyme classes require bimolecular oxygen, but no coenzyme, for activity. However, the enzymes differ in the mechanism by which they oxidize phenols. Laccases oxidize phenolic compounds to form their corresponding anionic free radicals, whereas tyrosinases form *o*-diphenols and subsequently release oxidized *o*-quinones (Sjoglad and Bollag, 1981). In an alkaline environment, the quinone products slowly polymerize through autooxidative processes. The laccases may prove to be the most useful of the phenoloxidases because they produce radicals which are very reactive and because, unlike the peroxidases, they do not require the presence of hydrogen peroxide.

OXIDATIVE COUPLING OF PHENOLIC AND ANILINIC COMPOUNDS WITH HUMUS

As previously discussed, many phenolic chemicals and their degradation products resemble naturally occurring humic acid precursors, and therefore they are often incorporated into humus during the humification process. Several studies have illustrated the incorporation of pesticides into humic material. Mathur and Morley (1978), for instance, have demonstrated the incorporation of the insecticide methoxychlor (1,1,1-trichloro-2,2-di-(4-methoxyphenol)ethane) into a humic acid analogue. Similarly, Wolf and Martin (1976) observed that 2,4-D (2,4-

dichlorophenoxyacetic acid) and chlorpropham (isopropyl 3-chlorophenyl carbamate) are incorporated into humic acid polymers.

The products formed during the cross-coupling of pollutants and humic acids are highly heterogeneous and complex and are therefore quite difficult to identify. In order to elucidate the mechanism by which contaminants are incorporated into humus and to identify the products formed, our laboratory studied the binding process through the use of model systems. Generally, different fungal laccases were incubated with various chlorinated phenols or aromatic amines in the presence of specific phenolic humic constituents. The hybrid products formed were separated by thin-layer chromatography or high-performance liquid chromatography and then isolated and characterized by mass and nuclear magnetic resonance spectroscopy. The compound 2,4-dichlorophenol (2,4-DCP), a degradation product of the herbicide 2,4-D, was shown to couple with humus-derived compounds such as orcinol, syringic acid, vanillic acid, and vanillin (Bollag et al., 1980). In further studies, phenols containing one to five chlorines (4-chlorophenol, 2,4- and 2,6-dichlorophenol, 2,3,6- and 2,4,5-trichlorophenol, 2,3,5,6-tetrachlorophenol, and pentachlorophenol) and various chloroanilines (4-chloroaniline, 3,4-dichloroaniline, and 2,4,5-trichloroaniline) were cross-coupled with syringic acid or guaiacol by the laccase from the fungus *Rhizoctonia praticola* (Bollag and Liu, 1985). Two types of cross-coupling products were formed: (1) quinonoid oligomers consisting of chlorophenols linked by ether bonds to orthoquinoline products of syringic acid; and (2) phenolic oligomers consisting of chlorophenols bound by ether linkages to decarboxylated products of syringic acid. The anilines were mostly coupled via imine linkages to quinone derivatives of the humus constituents.

In another study, the incorporation of ¹⁴C-labeled phenols into humus was examined under conditions which approximated the natural soil habitat (Sarkar et al., 1988). The research involved incubation of ¹⁴C-labeled-2,4-DCP with fulvic acid from stream water in the presence of different oxidoreductases. Upon dialysis of the reaction mixtures, about 65% of the initial radioactivity was firmly incorporated into fulvic acid. The coupling occurred over a wide pH and temperature range.

BIOLOGICAL CONSEQUENCES OF BINDING

Before the binding of contaminants to humus can be utilized for decontamination purposes, it will be necessary to fully investigate the stability of the bound complexes. If large quantities of a pollutant were released at a future

time, the accumulation of these compounds would pose a delayed environmental hazard. The stability of humus-bound contaminants has been demonstrated by several investigators. In one study, 3,4- dichloroaniline was applied to a German soil and 46% of the compound remained bound to the soil 2 years after treatment (Viswanathan et al.,1978). In a separate study, 83% of ¹⁴C-labeled-atrazine remained associated with the soil after 9 years; 50% of this residue represented bound material (Capriel et al.,1985).

The activity of microorganisms is believed to be the primary factor responsible for the release of bound residues. To study the release of bound pesticides, ¹⁴C-labeled-catechol and mono-, di-, tri-, and pentachlorophenols, bound to humic acid polymers, were incubated with microbial soil populations, and the release of radioactive compounds into the medium was monitored (Dec and Bollag, 1988). This study demonstrated that insignificant quantities of ¹⁴C-labeled compounds were released over a 13-week incubation period. Furthermore, this release was accompanied by a simultaneous mineralization of the bound material to ¹⁴CO₂. As might be expected, the release of bound xenobiotics differs with the type of binding. There appears to be a "surface" fraction of bound residue that is releasable, while the remainder is covalently bound to a "core" portion that is less accessible to microbial degradation. Overall, the available data indicate that the microbial release of bound contaminants occurs at an extremely slow rate. Once released, the contaminants can be mineralized or reincorporated into humus. Consequently, released residues are not expected to accumulate and should not pose a delayed health hazard.

CRITICAL FACTORS AFFECTING THE BINDING PROCESS

Given that the incorporation of contaminants into humus causes the elimination or reduction of their toxicity, future research should focus on methods for improving the binding process. It is clear that the binding of contaminants to humus depends on many environmental factors which vary with season, climate, soil type and agricultural practices. Because of this variability, it is necessary to evaluate the incorporation of each contaminant into humus under specific sets of environmental conditions. For example, the pH of the environment has been shown to play an important role in determining the efficiency of polymerization (Dec and Bollag, 1990). The phenoloxidases of many soil fungi have an optimum pH in the range of 4 to 7. Thus, in calcareous or acid soils, the activity of these enzymes may be reduced. In addition, the pH optimum has been found to depend on the

chemical structure of the substrate. The position and type of substituent group also influences the polymerization process. In general, transformation efficiency decreases with increasing molecular weight of the substituent group, e.g., methylphenols are transformed the most efficiently, followed by methoxyphenols, chlorophenols, and bromophenols. Furthermore, the transformation efficiency of phenols decreases with increasing number of chlorine atoms. Since the oxidative coupling process is influenced by such a large number of factors, and since these factors vary with the type of substrate and the enzyme employed, caution must be exercised in interpreting laboratory data and in extrapolating findings to the natural environment.

The use of cross-coupling techniques for soil decontamination purposes may be hindered by the relative inertness of some phenolic compounds to enzymatic action. However, it has been observed that the reactivity of some inert compounds can be enhanced by the addition of highly reactive substrates to reaction mixtures containing laccase. For instance, in the presence of a phenoloxidase or peroxidase and a humic monomer (e.g., guaiacol, vanillic acid etc.) the transformation of the herbicide bentazon or its metabolites was considerably enhanced (Kim et al., 1998). Thus, the applicability of the cross-coupling technique can be broadened by the use of co-polymerizing agents.

The efficiency of enzymatic coupling can also be enhanced through the use of enzymes bound to solid supports. According to our data (Sarkar et al., 1989), the immobilization of laccase on soil supports increases the thermostability and pH range of the enzyme, prevents its degradation by proteases, and increases its half-life. Overall, immobilized enzymes may prove to be more cost effective because they are biochemically more stable and are reusable.

APPLICATION OF NMR TO DETERMINE THE TYPE OF BINDING OF POLLUTANTS IN THE SOIL

Studies with ^{14}C -labeled pollutants have not clearly established whether a covalent interaction had occurred. It appears, however, that direct evidence for the formation of covalent bonds can be obtained by using ^{13}C -, ^{15}N - or ^{19}F -labeled chemicals in combination with ^{13}C -, ^{15}N - or ^{19}F -NMR spectrometry. The major advantage of this approach lies in the fact that pollutant molecules enriched with the ^{13}C , ^{15}N - or ^{19}F isotope generate more intensive NMR signals than those resulting from the natural abundance of these isotopes in the studied compound and soil. Changes in the location of these signals on the NMR spectra can provide

not only evidence for the occurrence of covalent binding but also information on the type of bond formed between a pollutant and humic material.

Our ^{13}C -NMR studies were carried out using two ^{13}C -labeled pollutants: 2,4-dichlorophenol, a degradation product of the herbicide 2,4-D (Hatcher et al., 1993), and cyprodinil, a new phenyl-pyrimidine amine fungicide manufactured by Novartis (Dec et al., 1997). 2,4-Dichlorophenol, labeled either in the C-1 or in the C-2 and C-6 position, was incubated for 2 hours with equal amounts of humic acid in the presence of a peroxidase. The NMR signals generated by the ^{13}C label demonstrated bonding between the two components through carbon-carbon, ester and phenolic ether linkages. To investigate the formation of covalent bonds under more natural conditions, cyprodinil, which was labeled either in the phenyl or pyrimidyl ring, was incubated for several months with fresh soil. After exhaustive washing with methanol, humic acid was isolated by extraction with 0.5 M NaOH. Humic acid was then purified by dialysis and/or silylated by treatment with trimethylchlorosilane to facilitate the ^{13}C -NMR analysis. The NMR signals generated by both the dialyzed and silylated samples indicated cleavage of the cyprodinil molecule between the aromatic rings and covalent binding of the phenyl and pyrimidyl moiety to humic acid.

Our results, especially those related to binding in the soil, confirm the great potential of using ^{13}C -labeled chemicals and ^{13}C -NMR spectrometry as an analytical technique for evaluating interactions between pollutants and soil components, particularly humic substances. Establishing the physical or chemical associations between pollutants and humic substances will provide essential data for understanding principles of bioavailability.

CONCLUSIONS

The incorporation of contaminants and/or their derivatives into humus occurs readily in nature. It has been suggested that this process can be exploited to immobilize and detoxify hazardous compounds. The binding of pollutants to humus has several important consequences: (1) the amount of compound available to interact with the biota is reduced; (2) the complexed products are less toxic than their parent compounds; and (3) binding restricts leaching of chemicals thus preventing contamination of ground water.

The use of enzymatic coupling for detoxification purposes raised concerns about the ultimate fate of bound pollutants. However, all available data indicate that, after xenobiotics are incorporated into soil, they are released at a very slow

rate and to a minimal extent. The gradual release should not pose a delayed health hazard because the released compounds can be mineralized to CO₂ or reimmobilized in the soil matrix.

Nevertheless prior to the practical application of this technique, extensive research is required to further analyze the accumulation, bioavailability and toxicity of bound pesticide residues in nature. Research should continue to focus on the development of new methods for maximizing the binding process, e.g., through the use of immobilized enzymes, abiotic catalysts or the addition of co-polymerizing agents. All current evidence indicates that the enzymatic or abiotic incorporation of xenobiotics into humus provides an efficient and cost-effective method for detoxifying hazardous pollutants.

LITERATURE

- Bollag, J.-M., M. Strynar, Mi-youn Ahn and J. Dec. 2002. Characterization of enzymatic or abiotic immobilization of xenobiotics in soil. In 'Soil Mineral-Organic Matter-Microorganism Interactions and Ecosystem Health. Ecological Significance of the Interactions Among Clay Minerals, Organic Matter and Soil Biota'. *Develop. Soil Sci.* Vol. 28B: 289-299.
- Bollag, J.-M. 1983. Cross-coupling of humus constituents and xenobiotic substances. In: *Aquatic and terrestrial humic substances* (Eds.: R. Christman and E. Gjessing), Ann Arbor Science Publishers, Inc., p. 127-141.
- Bollag, J.-M. and S.-Y. Liu. 1985. Copolymerization of halogenated phenols and syringic acid, *Pestic. Biochem. Physiol.*, 23: 261-272.
- Bollag, J.-M., S.-Y. Liu, and R. Minard. 1980. Cross-coupling of phenolic humus constituents and 2,4-dichlorophenol, *Soil Sci. Soc. Amer. J.*, 44: 52-56.
- Calderbank, A. 1989. The occurrence and significance of bound pesticide residues in soil, *Rev. Environ. Contam. Toxicol.*, 108: 71-103.
- Capriel, P., A. Haisch, and S. Kahn. 1985. Distribution and nature of bound (nonextractable) residues of atrazine in a mineral soil nine years after herbicide application, *J. Agric. Food Chem.*, 33: 567-569.
- Dec, J. and J.-M. Bollag. 2001. Use of enzymes in bioremediation. In: *Pesticide Biotransformation in Plants and Microorganisms: Similarities and Divergences*. ACS Symposium Series 777, J.C. Hall, R.E. Hoagland and R.M. Zablotowicz, eds., American Chemical Society, Washington, DC. pp. 182-193.

- Dec, J. and J.-M. Bollag. 1988. Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid. *Soil Sci. Soc. Amer. J.* 52: 1366-1371.
- Dec, J. and J.-M. Bollag. 1990. Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions, *Arch. Environ. Contam. Toxicol.*, 19: 543-550.
- Dec, J. and J.-M. Bollag. 1994. Use of plant material for the decontamination of water polluted with phenols, *Biotechnol. Bioeng.* 44: 1132-1139.
- Dec, J., K. Haider, A. Benesi, V. Rangaswamy, A. Schäffer, U. Plücken and J.-M. Bollag. 1997. Analysis of soil-bound residues of the ^{13}C -labeled fungicide cyprodinil by NMR spectroscopy. *Environ. Sci. Technol.* 31, 1128-1135.
- Hatcher, P., J. Bortiatynski, R. Minard, J. Dec, and J.-M. Bollag. 1993. Use of high-resolution ^{13}C NMR to examine the enzymatic covalent binding of ^{13}C -labeled 2,4-dichlorophenol to humic substances, *Environ. Sci. Technol.*, 27: 2098-2103.
- Hatzinger, P. and M. Alexander. 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol.*, 29: 537-545.
- Helling, C. and A. Krivonak. 1978. Physicochemical characteristics of bound dinitroaniline herbicides in soil, *J. Agric. Food Chem.*, 26: 1156-1163.
- Katan, J. and E.P. Lichtenstein. 1977. Mechanisms of production of soil-bound residues of [^{14}C] parathion by microorganisms, *J. Agric. Food Chem.* 25: 1404-1408.
- Khan, S. 1978. In: *Soil organic matter* (Eds.: M. Schnitzer and S. Khan), Elsevier Scientific Publishing Co., New York.
- Kim, J.-E., C.-J. Wang and J.-M. Bollag. 1998. Interaction of reactive and inert chemicals in the presence of oxidoreductases: Reaction of the herbicide bentazon and its metabolites with humic monomers. *Biodegradation.* 8: 387-392.
- Klibanov, A., B. Alberti, E. Morris, and L. Felshin. 1980. Enzymatic removal of toxic phenols and anilines from waste waters, *J. Appl. Biochem.*, 2 414-421.
- Koskinen, W.C. and S.S. Harper. 1990. The retention process: Mechanisms. In: *Pesticides in the soil environment: Processes, impacts, and modeling* (Ed.: H.H. Cheng), Soil Sci. Soc. of Am., Inc., Wisconsin, p. 51-77.
- Maloney, S., J. Manem, J. Mallevalle, and F. Fiessinger. 1986. Transformation of trace organic compounds in drinking water by enzymatic oxidative coupling, *Environ. Sci. Technol.*, 20: 249-253.

- Mathur, S. and H. Morley. 1978. Incorporation of methoxychlor-¹⁴C in model humic acids prepared from hydroquinone, *Bull. Environ. Contam. Toxicol.*, 20: 268-274.
- Park, Jong-Woo, J. Dec., J.-E. Kim and J.-M. Bollag. 2000. Transformation of chlorinated phenols and anilines in the presence of humic acid. *J. Environ. Qual.* 29: 214-220.
- Pignatello, J., 1989. In: Sorption dynamics of organic compounds in soils and sediments (Eds.: B. Sawhney and K. Brown), Reactions and movement of organic chemicals in soils, Spec. Publ. No. 22, Soil Sci. Soc. Am., Inc., Madison, Wisconsin, p. 45-80.
- Roberts, T. 1984. Non-extractable pesticide residues in soils and plants, *Pure Appl. Chem.*, 56: 945-956.
- Sarkar, J. M., A. Leonowicz and J.-M. Bollag. 1989. Immobilization of enzymes on clays and soils, *Soil Biol. Biochem.* 21: 223-230.
- Sarkar, J., R. Malcolm, and J.-M. Bollag. 1988. Enzymatic coupling of 2,4-dichlorophenol to stream fulvic acid in the presence of oxidoreductases, *Soil Sci. Soc. Am. J.*, 52: 688-694.
- Sjoblad R. and J.-M. Bollag. 1981. Oxidative coupling of aromatic compounds by enzymes from soil microorganisms. In: *Soil biochemistry*, vol. 5 (Eds.: E. Paul and J. Ladd), Marcel Dekker Inc., New York, 113-152.
- Viswanathan, R., I. Scheunert, J. Kohli, W. Klein, and F. Korte. 1978. Long-term studies on the fate of 3,4-dichloroaniline-¹⁴C in a plant-soil system under outdoor conditions, *J. Environ. Sci. Health B*, 13: 243-259.
- Wang, T., P. Huang, C.-H. Chou, and J.-H. Chen. 1986. Interactions of soil minerals with natural organics and microbes, Spec. Publ. No. 17, Soil Sci. Soc. Am., Inc., Madison, Wisconsin, p. 251-281.
- Wolf, D. and J. Martin. 1976. Decomposition of fungal mycelia and humic-type polymers containing carbon-14 from ring and side-chain labeled 2,4-D and chlorpropham, *Soil Sci. Soc. Amer. Proc.*, 40: 700-704.

국문요약

기존의 오염물질을 제거하는 많은 화학적-물리적 정화 방법은 고비용과 오랜 시간을 요구하는 처리 과정 등의 단점을 갖고 있는 경우가 많았다. 따라서 흙과 수(水)환경내로 유입된 오염물질을 빠른 시간 내에 제거할 수 있는 대안이 요구 되었다. 흙에 유출된 화학 물질 중 상당량은 흙에 의해 격리, 구속 되고 이로 인해 일단 구속된 오염물질은 물과 유기 용매에 의해서도 잘 추출되지 않는 것으로 보고 되고 있다. 이러한 흙에 의한 오염물질의 비유동성(immobilization) 과정은 오염물질의 제거 기술의 대안으로 평가 될 수 있다. 기존 연구자들의 연구 결과, 화학적 혹은 물리적 반응 작용을 통해 오염물질을 흙을 구성하는 물질에 구속할 수 있음이 증명되었다. 이러한 과정 중 환경적 측면에서 볼 때, 화학적 반응이 더 우수하다 할 수 있다. 이는 강한 공유결합(covalent bonds)으로 연결될 경우 미생물의 활동이나 화학 처리로도 이를 분리하기 어렵기 때문이다. 리그닌(lignin) 분해에서 발생하는 휴믹(humic) 물질 등이 안정된 화학적 연결을 통해 흙 매질 내에 오염물질과 결합하는 대표적 물질이다. 인위적으로 제조된 많은 화학물질은 자연적에서 발생하는 휴민산 발생원(humic acid precursors)과 닮았다. 따라서 화학물은 부식과정(humifications process)동안 부식토(humus) 내로 병합(incorporate)되어 진다. 일단 이렇게 구성된 결합체는 생물체와 오염물질과의 반응을 방지하여 오염물질로 인한 생물체로의 독성을 감소시키는 역할을 하게 된다. 본 논문에서는 이러한 흙의 유기물(organic matter)와 오염물질과의 결합체에 대한 평가로서 다음의 항목에 대한 고찰이 이루어 져야 함을 강조하였다.

- (a) 결합체에서 생물체(biota)와의 반응에 의해 오염물질은 감소되는가?
- (b) 모(parent) 화합물과 비교하여 복합체 생성물(complexed products)이 얼마나 덜 유독한가?
- 그리고 (c) 지하수 오염이 오염물질의 유동성 구속에 의해 얼마나 감소되는지?

Reactions Between Xenobiotics and Humic Material

오염물질의 화학적 결합의 예로 폴리페놀 산화효소(polyphenol oxidases)에 의한 생물학적 기작과 점토와 금속 산화물에 의한 비생물학적인 기작에 의하여 형성될 수 있다. 이러한 자연 발생적인 과정을 통해 오염물질의 유독성은 제거되는 것으로 판단할 수 있다. 반면 일반적으로 특별한 미생물 배양 혹은 식물 물질로부터 얻은 효소를 포함하는 개량된 흙은 오염물질의 병합(incorporation) 기작을 향상시키는 반면, 이러한 개량 과정이 없는 기존 토양에 토착하는 효소만으로는 오염물질의 분해에는 만족스러운 결과를 얻을 수 없었다. 반응 기작을 돕는 효소의 촉매 효과는 휴믹 물질로의 오염물질의 결합 범위, 그리고 결합으로 생성되는 복합체(complexes) 구조의 해석을 통해 평가할 수 있다. 페놀 화합물의 경우 반데르 발스력, 전이 착화물(charge-transfer complexation), 수소결합, 소수성 반응 등에 의하여 흙 콜로이드에

흡착되게 된다. 흡착(adsorption)의 성향과 흡착 강도는 화학물질의 종류와 분자의 구조에 의해 좌우된다. 흡착은 가역 반응(reversible process)이므로 흡착된 화학물질은 다시 방출될 수 있으며, 방출된 화학물질은 생물체와 반응할 수 있다. 그러나 장기간 흡착된 잔여물(adsorbed residues)은 추출 및 분해에 대한 저항성이 큰 것으로 보고 되고 있다. 이러한 저항성은 지속적이며 느리게 진행되는 오염물질이 부식토내로 병합(incorporation)되는 과정의 결과로 판단되어 진다. 오염물과 휴믹 물질과의 공유결합에 의한 결합체는 "*bound residues*"라고 불리우며, 이는 산과 염기에 강한 저항력이 있을 뿐 아니라 열 처리, 그리고 미생물의 분해에도 저항력이 강한다(Helling et al). 산화 커플링(oxidative coupling)은 부식 과정(humification process)에서 가장 주요한 화학 반응이다.

Oxidative Coupling of Phenolic and Anilinic Compounds with Humus

일부 연구자들은 살충제가 휴믹 물질 내로의 병합(incorporation) 과정을 설명하고자 하였다(Mathur and Morley, 1978; Wolf and Martin, 1976). 오염물질과 휴민산의 cross-coupling 과정에서 생성되는 생성물은 매우 비균질하고 복잡하기 때문에 이를 규명하기가 어렵다. 오염물질이 부식토(humus) 내로의 병합 기작을 규명하고, 형성된 생성물을 분석하기 위해 실험실에서 모델 시스템을 이용하여 결합 과정을 연구하였다. 일반적으로 다른 fungal laccases 를 특정 페놀 부식 성분(phenolic humic constituents)이 있는 상황에서 다양한 클로리네티드 페놀 혹은 방향족 아민과 함께 배양하였다. 합성된 생성물은 thin-layer chromatography 와 HPLC 로 분리하고, 질량 MRS(magnetic resonance spectroscopy)와 핵 MRS 를 이용하여 분석하였다. 이를 통해 반응물질과 생성물질과의 관계를 규명하고자 하였다.

Biological Consequences of Binding

Humus-bound 오염물질의 안정성을 기존 연구에서 증명되었는데, 그 중 3,4-dichloroaniline 과 German soil 과의 반응 연구에서는 반응 후 2년이 지난 뒤 약 46%의 화합물이 흙에 남아있었다. 또 다른 연구에서 흙과 결합 후 9년이 지난 후에 14C-labeled-atrazine 의 83%가 남아 있었고, 이들 잔여물 중 50%가 bound material 로 판명되었다(Capriel et al. 1985). 오염물질의 bound residues 의 유출(release)에 가장 중요한 인자는 미생물의 활동인 것으로 알려져 있다. 따라서 이러한 미생물 활동에 의한 오염물질의 유출에 대한 연구가 진행되었었다. 그러나 관련된 연구 결과 미생물이 반응할 수 있는 부분은 거의 bound residue 의 겉 surface" 부분이며, 공유 결합의 핵심(핵 ore")부분은 미생물에 의한 분해가 거의 이루어지지 않았다. 또한 미생물에 의한 오염물의 유출은 매우 느린 속도로 진행되며, 또한 일단 유출된 오염물질의 경우, CO₂ 로 광물화(mineralize)되거나 부식토에 의하여 재병합(reincorporation)되는 것으로 판명되었다.

Critical Factors Affecting The Binding Process

부식토와 오염물질의 결합은 많은 외적 환경 요인에 영향을 받을 것으로 보이는데, 이러한 요인에는 계절적 변화, 기후, 흙의 종류, 농사 활동 등이 있다. 이러한 변화적 요인으로 인해, 오염물질과 부식토와의 반응 특성을 규명하기 위해서는 특정 환경 조건에서 각 오염물질과 부식토의 병합 과정을 평가할 필요가 있다. 예를 들어, pH는 polymerization의 효율을 결정하는 중요한 역할을 한다. 많은 soil fungi의 phenoloxdases의 최적 pH는 4-7 사이이다. 따라서 석회질 흙이나 산성 흙에서는 이들 효소의 활동이 감소된다.

Conclusion

지금까지 기술한 바와 같이 오염물질의 부식토(humus)내로의 병합(incorporation) 작용을 통해 유동성을 억제함으로써 오염물질의 위험성을 감소시킬 수 있음을 여러 연구 결과를 통해 기술하였다. 현재까지 발표된 결과로 볼 때, 부식토(humus)로의 효소 및 무생물에 의한 오염물질의 병합은 유독한 오염물을 무독화(detoxifying) 하는 저비용의 효율이 우수한 기법으로 평가할 수 있다. 그러나, 본 기술을 현장에 적용하기 위해서는 binding process를 극대화기 위한 방법 등에 대한 집중적인 연구가 계속 진행되어야 할 것이다.