Experientia 39 (1983), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Incorporation of xenobiotics into soil humus

by J.-M. Bollag and M.J. Loll

Laboratory of Soil Microbiology, Department of Agronomy, The Pennsylvania State University, University Park (Pennsylvania 16802, USA)

1. Introduction

The binding of pollutants to soil organic matter is a cause of some ecological concern. Many industrial and agricultural chemicals are structurally similar to humus constituents and, therefore, may be incorporated into soil organic matter during humification. Once bound to humus some pollutants are apparently detoxified. Several researchers have observed that the phytotoxicity of herbicides declines in soils which have a high organic matter content[92,97,99,114]. Katan et al.[56] found that parathion residues in soil had no effect on fruit flies. However, it is not known if detoxication is permanent or temporary. Other studies indicate that xenobiotics bound to humic substances can later be released, thus posing a delayed health hazard. Microorganisms[52,66,79] and earthworms[34] can free these pollutants, which may subsequently be taken up by plants and agronomic crops[33,34,49,63,113,116,118]. In addition, the incorporation of man-made molecules into organic matter may affect soil structure and various physicochemical, microbiological and biochemical soil processes.

Incorporated xenobiotics, also known as bound residues, are compounds foreign to soil which can not be extracted from humus by ordinary analytical methods. The term 'bound residue' is, to a large extent, defined by the extraction technique used. Since solvents vary in their extraction efficiencies, a xenobiotic which is inextractable using one method may be released when other solvents or reaction conditions are employed. As Kaufman has discussed in his introduction to a symposium on the subject, a precise definition of a bound residue is difficult to formulate[58].

Incorporation into humus depends upon the type of xenobiotic introduced into soil and upon its subsequent transformation by microorganisms and abiotic factors. Certain man-made compounds are degraded to reactive intermediates (e.g., anilines and phenols) which are more likely to bind to soil organic matter than the parent chemicals. Binding may be catalyzed by microbial enzymes, some of which have been implicated in coupling reactions between pollutants and humus constituents[10, 15, 17]. Microbial populations can further influence incorporation by changing the physical or chemical environment of soil through their metabolic activities. Microbial metabolism can affect soil pH, redox conditions, oxygen content and many other factors. In addition, abiotic soil components, such as metals and clays, seem to be important catalysts in the humification process[10,121].

Xenobiotics are bound to humic substances by ionic or covalent bonding. They may also adsorb to humic substances by Van der Waals attractions, hydrogen bonding, charge transfer and hydrophobic bonding[62]. The type of bonding which predominates is determined by the soil environment and by the chemical properties of the xenobiotic and the humus.

In order to understand how xenobiotic-humus complexes are formed, it is necessary to briefly review the chemistry of humus and the process of humification.*

2. Humus and humification

Humus is defined by Brady as a complex and rather resistant mixture of brown or dark brown amorphous, colloidal substances synthesized from the tissues of various organisms[22]. It is produced from the remains of decomposing plants, animals and microorganisms. Humus' unique chemical and physical properties make it important for soil fertility and it serves as a source of nitrogen, phosphorus, sulfur, and micronutrients for plants. In addition, humus increases cation exchange capacity, aeration, percolation, and water-holding capacity and helps to prevent soil erosion[110].

Humic material is usually divided into 3 fractions: 1. *humic acid,* which is soluble in dilute alkali and precipitates upon acidification of the alkaline extract, 2. *fulvic acid,* which is soluble in acid and base, and 3. *humin,* which is insoluble in both acid and dilute alkali. All of these fractions are thought to have the same fundamental structure, for which Stevenson[110] has proposed a model. He theorized that humic molecules are large aromatic polymers which occur as micelles in nature. The polymers are made up of nitrogen heterocycles, quinones, phenols, and benzoic acids. They have carboxyl, hydroxyl, carbonyl, and thiol groups which may act as binding sites for carbohydrates and amino acids. Humic compounds also have aliphatic moieties, some of which are hydrophobic. It is interesting to note that soil organic matter contains stable free radicals in relatively high concentrations[109].

There are differences, as well as similarities, in the chemical and physical properties of the various humic components. Fulvic acid contains more oxygen than

---

* Chemical names of various xenobiotic compounds are listed in table 3.

humic acid but less carbon. Most of the oxygen in fulvic acid is in functional groups such as hydroxyls, carbonyls, etc. In humic acid a large proportion of the oxygen is found in the 'core' structure in ether and ester linkages. Fulvic acid has more carboxyl groups than humic acid and is, therefore, more acidic[32, 102]. Of the three fractions, fulvic acid has the lowest molecular weight; typical extracts range from 270 to 2100 daltons[102]. Humic acids are larger, ranging from 1400 to 1,000,000 daltons[112]. Humin appears to be similar to humic acid in its analysis and properties, but it differs from humic acid in its insolubility, which is thought to be caused by its complexation with inorganic materials from soil[102].

Phenols are among the major constituents of soil organic matter. They may account for up to 30% of the weight of the humic polymer. A variety of phenolic compounds have been isolated from humic substances and some of them are listed in table 1. Quinones, which are derived from phenols, also make up a sizable portion of the soil organic matter. Mathur[78] found that benzoquinone and 2-methyl-1,4-napthoquinone accounted for 10 and 15%, respectively, of his humus sample. Other hydrocarbons include benzenecarboxylic acids[103], polycyclic compounds[112], alkanes, fatty acids, ketones, phthalates[112] and hexose and pentose carbohydrates[103].

Humus contains organic nitrogen as amino acids, amino sugars, or as heterocycles. Amino acids may account for approximately 15% of some humus substances. Part of this α-amino nitrogen could be in the form of peptides and proteins[74]. In certain soils purines and pyrimidines are important nitrogen monomers[29].

Four hypotheses have been advanced to explain how humification takes place[31]. The *plant alteration hypothesis* is the oldest of these. Waksman's explanation of humus as a ligno-protein 'nucleus' was probably one of the first models of this type[119]. This premise states that lignin and other resistant plant materials in soil undergo only slight changes to form high molecular weight humic acids. The humic acids gradually are decomposed to fulvic acids, water, and carbon dioxide. In the *microbial synthesis hypothesis* humus is thought to be produced by microorganisms. Certain bacteria and fungi synthesize intracellular high molecular weight humic polymers in the course of normal metabolism. The organisms derive energy for metabolism from plant remains. When these microbes die, they release the polymers into soil. The *chemical polymerization hypothesis* is a variation on this theme. Soil microbes produce amino compounds and phenols and then discharge them into the environment. Once in soil, these monomers polymerize to form humus. According to the *cell autolysis hypothesis* both plants and microbes contribute to humification. These organisms die, autolyze, and their residues (which include sugars, amino acids, phenols, benzenecarboxylic acids, etc.) polymerize. It is probable that all four of these processes occur simultaneously in soil, although which one predominates is not known.

Table 1. Organic compounds originating from humus

Acids	Aldehydes	Polyphenols
2,4-Dihydroxybenzoic acid	*p*-Hydroxybenzaldehyde	Catechol
Ferulic acid	Syringaldehyde	Orcinol
Gallic acid	Vanillin	Phloroglucinol
m- and *p*-Hydroxy benzoic acids		Pyrogallol
Protocatechuic acid		Resorcinol
Syringic acid		2,4-Dihydroxytoluene
Syringylpropionic acid		2,6-Dihydroxytoluene
2,3,4-Trihydroxybenzoic acids		
Vanillic acid		

The preceding discussion points out the importance of polymerization mechanisms in humification. As will be shown in the next section, these mechanisms also play a role in the incorporation of man-made chemicals into humus. One of the most important polymerization reactions is oxidative coupling. This is a free radical reaction which links phenols, anilines, amino acids, or other compounds into polymers. It is brought about by plant and microbial enzymes and also by metals and clays. Many soil microorganisms have phenoloxidases which can catalyze oxidative couplings[106]. These organisms have been shown to form oligomers from typical humus monomers. Bollag et al.[16] and Liu et al.[73] demonstrated that a laccase from the soil fungus *Rhizoctonia praticola* could polymerize vanillic and syringic acids. The fungus *Hendersonula toruloidea* also produced humic-type polymers from a number of different phenols[77].

Humus itself might act as an agent in its own formation[112]. Free radicals in the 'core' structure may oxidize a variety of compounds.

Inorganic compounds can cause oxidative coupling reactions. Clays have the ability to polymerize phenols[120, 121] and benzene[84]. Various metals have similar oxidative coupling activities; salts of copper, lead, silver, and zinc can link phenols[88] and Larson and Hufnal[68] used suspensions of manganese dioxide, cupric oxide, and zinc oxide to polymerize catechol.

Finally, oxidative coupling reactions occur spontaneously. Certain reactive phenols autoxidize in the presence of oxygen at neutral and alkaline pH[88]. These spontaneous ractions may incorporate nonphenolic compounds into humic polymers. Polyphenols and quinones bind to amino acids, amino sugars, and peptides by way of autoxidative processes[41]. Bondietti et al.[19] speculate that nucleic acids and various agrochemicals may undergo similar spontaneous transformations with quinones.

It is obvious that both biological and nonbiological mechanisms are involved in humification. At present, however, the respective importance of each is not known.

3. Reactions between xenobiotics and humic materials

The biological and physicochemical mechanisms by which various chemical groups of xenobiotics bind to humus are discussed in this section. While the roles of both biological and nonbiological agents in these processes are included, the main focus, where applicable, is on biochemical reactions.

Reactions of phenols

Xenobiotic phenols are common pollutants. They are found in soil as pesticides, industrial wastes, or their breakdown products. These compounds include pentachlorophenol, the widely used wood preservative, and degradation intermediates of the pesticides parathion[86], fenitrothion[108], carbaryl[1,12], cypermethrin[101], oxadiazon[2], 2,4-D and MCPA[75]. Once in soil phenols may bind to soil organic matter by oxidative coupling. A study by Kazano et al.[59] indicated that 1-naphthol, a carbaryl derivative, may be covalently bound to humic and fulvic acids by this process. Wolf and Martin[126] found that 12% of the radioactivity from ^{14}C-ring labelled 2,4-D was incorporated into humic-like polymers produced by fungi. They suggested that incorporation took place as phenolic derivatives of the pesticide polymerized with the humic substances.

Microbial enzymes have been implicated in the production of bound phenolic residues. Bollag et al.[15] studied the enzymic polymerization of humic monomers with 2,4-dichlorophenol, a breakdown product of 2,4-D. They found that an extracellular laccase from the fungus *Rhizoctonia praticola* coupled the dichlorophenol with syringic acid, vanillic acid, vanillin and orcinol, all of which are constituents of humus. The hybrid compounds obtained were dimers, trimers, tetramers, and pentamers. The fungal enzyme coupled pentachlorophenol and syringic acid to form several hybrid oligomers[13]. By linking xenobiotics to each other the enzyme could also form 'novel' humic-type polymers. The laccase dimerized 2,4-dichlorophenol[82], and some of the products were dichlorophenoxy-benzoquinones, molecules in which chlorobenzoquinones are bound to chlorobenzenes by ether linkages. Under acidic conditions phenoxyquinones such as these may cyclize to form dioxin. In separate experiments the enzyme polymerized 2- and 4-chlorophenol, 4-chloro-2-methylphenol and 4-bromo-2-chlorophenol, which are intermediates of 2- and 4-chlorophenoxyacetic acid, MCPA, and 4-bromo-2-chlorophenoxyacetic acid, respectively[18]. When Sjoblad et al.[107] incubated 1-naphthol, or dihydroxynaphthols with the *R. praticola* enzyme, a number of oligomers resulted. Pentameric products and higher molecular weight compounds were obtained from 1-naphthol. Xenobiotic hybrids were produced by adding laccase to a mixture of 2,4-dichlorophenol and 4-bromo-2-chlorophenol[14]. Nitrophenols, which result from the degradation of parathion and related pesticides, were not oxidized by the laccase[105]. The electron-withdrawing nitro groups probably prevent free radical formation.

It should be noted that the synthesis of humic-type polymers from xenobiotics can present problems for residue analysis and public health. These foreign polymers complicate the identification of pollutants in soil and have unknown toxicities. As in the case of certain dioxins, xenobiotic oligomers can be more dangerous than their precursor molecules.

Reactions of aromatic amines

Aromatic amines and their derivatives are used extensively in industry and agriculture. A large number of currently applied pesticides such as phenylureas, phenylcarbamates, acylanilides, dintroaniline herbicides, and certain fungicides contain halogen- and alkyl-substituted aniline rings. Aromatic amines are usually reactive compounds and appear to bind easily to soil organic matter. An oxidative coupling reaction seems responsible for binding, whereby anilines are oxidized to arylamino radicals which can be linked with phenols[91].

However, various researchers have noted the difficulty of recovering aromatic amines from soil[5,11,27,117]. As was first demonstrated with 3,4-dichloroaniline, an intermediate of the herbicide propanil, it is very difficult to extract this chemical from soil using organic solvents or by acid and alkaline hydrolysis[5,27]. Hsu and Bartha observed that about half of the humus-bound ^{14}C-labeled 3,4-dichloroaniline could be released by hydrolysis, while the remainder was liberated only by combustion[53,54]. They concluded that anilines are associated with humus by 2 types of bonds: the weaker, hydrolyzable ones could be anil (imine) or anilinoquinone linkages, while the stronger bonds could be explained by the incorporation of amino groups into heterocyclic ring structures.

Anils form when anilines react with humic carbonyl groups such as those of benzaldehyde moieties. Under aerobic conditions inextractable dinitroanilines have been found predominantly in the carbonyl-rich fulvic acid fraction of soil[48]. Thermal analysis showed that most of these residues were associated with carboxyl or phenolic portions of the fulvic acid molecule. When humate carbonyl groups are blocked by previous reaction with ammonia or reduced by sodium borohydride, aniline binding is decreased[90]. In the same study, however, it was found that imine formation is rapid and reversible whereas anilinoquinones are produced in a slower, irreversible reaction. Work with methylcatechol-aniline humic analogues suggests that anilines are more likely to bind to quinones to form anilinoquinones and less likely to bind to carbonyls to form imines[128].

It also was shown that 2,6-diethylaniline, 3,4-dichloroaniline, and 4-chloroaniline reacted with phenolic humus constituents in the presence of a fungal laccase[17]. The same enzyme could link 2,4-dichlorophenol to various halogenated anilines[72]. In both cases the resulting products were imines or anilinoquinones. No reaction occurred when only the anilines and the fungal laccase were incubated together. Further experimentation proved that the anilines reacted with quinones produced by the fungal laccase[72,82].

The reactions of anilines with quinones are influenced by the substituent groups on the aniline ring. Reaction rates for some representative anilines with benzoquinone decreased as follows: 4-methylaniline > aniline > 4-chloroaniline > 3,4-dichloroaniline > *N*-methylaniline > 2-chloroaniline > 2,5-dichloroaniline[90].

The nonhydrolyzable residues probably result from the formation of heterocyclic phenoxazine and phenazine rings[54]. These rings bind the nitrogen atom of the aniline into a very stable structure which is resistant to acids and bases. However, there is a possibility that these anilines can be released into soil by microbial enzymes.

At high concentrations arylamino radicals react with each other to form azo products. It is important to monitor these compounds in soil because they are potentially toxic. The condensation reactions which produce azo compounds from anilines can be catalyzed by plant and microbial enzymes. A peroxidase and an aniline oxidase from the fungus *Geotrichum candidum* could dimerize most anilinic substrates except those having strong electron withdrawing groups, i.e., nitroanilines[20]. Condensation may occur directly between 2 anilines or it may take place when microorganisms transform anilines to nitrosobenzenes[7]. The nitroso intermediates could then spontaneously react with anilines.

Nitrobenzenes and other nitrogen-containing xenobiotics also may bind to soil humus. Many of these chemicals are converted to anilines which subsequently react with organic matter. Table 2 lists a number of compounds which are reduced to aromatic amines in soil. In some cases reduction appears to be biologically induced. Katan et al.[57] reported that microorganisms change parathion to its amino derivatives. Wheeler et al.[125] assumed that the degradation of trifluralin to anilines in soil was brought about by biological agents. Thermophilic microorganisms transform TNT, a common pollutant at munitions sites, to various nitroanilines[55].

While biological mechanisms appear to play a role in the binding of anilines to soil organic matter, there is evidence that nonbiological processes are also involved. Inextractable aniline products form in sterile as well as nonsterile soils[11,27,53,95]. Various condensation products have been detected in autoclaved soil treated with aniline[95]. The agents which promote nonbiological coupling have not been determined, but free radicals in humic material do not appear to be responsible[53].

Reactions of phenylureas

Phenylureas are broad-spectrum herbicides which are introduced into soil through direct or foliar applications. They may be incorporated into soil organic matter as parent compounds or as their degradation products, some of which are anilines. For the most part, intact phenylureas are probably bound through nonbiological processes. Phenylureas evidently do not undergo oxidative coupling or cation exchange in their adsorption to humus[104], although other types of substituted ureas are bound by the latter mechanism[122]. It appears that phenylureas attach to humic substances by way of hydrogen bonding, Van der Waals forces, and charge transfer interactions[43,104], and, in some cases, hydrophobic attractions may be involved[25]. Inorganic ions attached to humic functional groups can complex with phenylureas at their carbonyl or amine positions. The amount of pesticide complexed depends on the pesticide and on the ion; for linuron adsorption to peat increased in the order of $Ca^{2+} < Ni^{2+} < Cu^{2+} < Fe^{3+} < Ce^{4+}$ and was independent of pH[44]. However, other work contradicts these findings. When phenylureas were adsorbed to the exchange cations of humic acids, the degree of adsorption was the same with Fe^{3+}, Al^{3+}, or Cu^{2+}[35]. According to the same report adsorption increased as pH decreased, probably due to an increase in hydrogen bonding between the pesticides and the soil colloids. Discrepancies between these two studies may be due to differences in the organic adsorbents used.

Table 2. Binding in soil of various amino-derivatives of xenobiotic compounds

Pesticide	Amino-derivative	Amount bound in soil	Reference
Benefin	Mixture of aromatic amines	32% (352 days)	39
2,6-Dichloro-4-nitroaniline	Not determined		117
Flamprop-isopropyl	3-Chloro-4-fluoroaniline	7% (10 weeks)	51
Isopropalin			36
Oryzalin	Unidentified aromatic amines	30% (1 year)	38
Parathion	Aminoparathion and *p*-aminophenol	17% and 46% (28 days)	56, 70
Pentachloronitrobenzene	Pentachloroaniline	41% anaerobic (24 days) 69% aerobic (24 days)	87
Phosalone	'Aniline metabolite'	80% (84 days)	3
Propanil	3,4-Dichloroaniline	73% (20 days)	5
Trifluralin	Various amino-derivatives	43% (12 months)	37, 98
2,4,6-Trinitrotoluene (TNT)	Amino- and diamino-nitrotoluenes	22% (91 days)	55

Reactions of triazines

Triazines are among the most widely used herbicides. They are heterocyclic nitrogen compounds which can be protonated with relative ease, thus obtaining a positive charge in the process. As opposed to anilines and phenols, triazines do not polymerize with humus. Their ability to become positively charged implies that cation exchange may be the primary mechanism by which triazines bind to soil organic matter; however, this is still a point of some contention. At certain soil pH values triazines would not be protonated and hydrogen bonding or Van der Waals forces might be responsible for adsorption. There is abundant evidence for ionic and nonionic types of bonding. Carringer et al.[25] were able to displace prometryn from peat using 1 N $CaCl_2$. This suggested that cation exchange occurred between the triazine and the peat. Infrared studies performed by Senesi and Testini[104] implied that *s*-triazines largely react with humic carboxyls by ion displacement. However, they did not rule out the possibility that other interactions can occur between these pesticides and soil organic matter. Results by Weber et al.[123] also implicate ionic processes in the incorporation of triazines.

Hayes[46], on the other hand, emphasized hydrogen bonding as an adsorption mechanism. In his review he pointed out that, when saturated with hydrogen ions at low pH, humic acids provide good environments for this type of bonding to occur. Hydrophobic attractions may also bind triazines. Atrazine, for example, adsorbs to long chain alkyl groups[43]. Alkanes, fatty acids, and other hydrophobic moieties in humus may act as binding sites for hydrophobic xenobiotics. According to Khan[64] the incorporation of ^{14}C-prometryn into humus is the result of hydrophobic interactions and molecular sieving. Humic polymers may bind together to form a molecular sieve. This structure would have spaces of different molecular size which could trap various sorts of molecules and bind them hydrophobically. Khan suggested that prometryn molecules become bound within the pores of the humic structure.

The adsorption of triazines also depends on the substituent groups of the triazine. An increase in the number of alkyl groups at the 4,6-amino positions of the triazine ring promotes adsorption[123]. Alkylation increases the basicity and hydrophobicity of these pesticides, thus enhancing their reactivity with humus.

It should be noted that ionic and physicochemical reactions occur spontaneously in soil and are not mediated by catalysts. The role of microorganisms in binding triazines and phenylureas is limited to modifying these chemicals prior to their adsorption on humic colloids. There is no evidence for the enzymic formation of bound triazine or phenylurea residues in humus.

Reactions of bipyridylium herbicides

Diquat and paraquat are the best known members of this class of compounds. These 2 nonspecific herbicides are used extensively against terrestrial and aquatic weeds. They are quarternary ammonium salts and easily dissolve in water, in which they ionize to divalent cations. They do not volatilize and are stable at neutral and acid pH.

Several studies indicate that diquat and paraquat bind to soil organic matter primarily by ion exchange. Khan[61] saturated humic acid with hydrogen and various tri- and divalent ions and then equilibrated it against solutions of diquat and paraquat. As the herbicides were adsorbed, these ions were displaced from the humic colloid. The amount of herbicide adsorbed was contingent upon the bound cation. Adsorption decreased as follows: $Al^{3+} < Fe^{3+} < Cu^{2+} < Ni^{2+} < Zn^{2+} < Co^{2+} < Mn^{2+} < H^{+} < Ca^{2+} < Mg^{2+}$. As the herbicides were added to humic acid, the IR spectra of the latter changed. For example, the 1720 cm^{-1} band (protonated carboxyl) shifted to 1610 cm^{-1} (ionized caboxyl). Carboxylate groups provide humus with some of its exchange capacity and this finding indicated that the pesticides were reacting with humus by cation displacement. Adsorption has been shown to be temperature independent[23], which is also typical of an exchange mechanism. Adsorption and desorption experiments with paraquat on humate provide further support for this theory[24].

Other physicochemical forces are involved in the formation of bound bipyridylium residues, although to a lesser degree than cation exchange. Charge-transfer reactions are one way in which diquat and paraquat bind to humic substances[61]. In their report on the adsorption behavior of paraquat on humic acid, Burns et al.[24] also mention the possible contributions of hydrogen bonding and Van der Waals forces to the process.

Reactions of hydrophobic hydrocarbons

A number of reports show that water insoluble compounds like alkanes, phthalates, and chlorinated pesticides form complexes with humus. While this may seem surprising in light of their immiscibility with the soil solution, it appears that hydrophobic hydrocarbons can react with nonpolar components of soil organic matter. These reactions could influence the movement of xenobiotics in soil and water.

One group of environmentally dangerous hydrocarbons are the polychlorinated biphenyls (PCB's) which enter soil from industrial pollution, sludge amendments, and herbicide applications. Recent work shows that they bind to humus in the soil environment. PCB adsorption has been related to organic matter content[28,50] and the toxicity of the PCB herbicide Aroclor 1254 decreases in the presence of organic matter[114].

The exact mechanism by which PCB's are incorporated into humus is not known. Moza et al.[85] speculate that PCB's in soil are metabolized to phenols which polymerize to form bound residues. However, it is more commonly accepted that these xenobiotics are bound to humic material noncovalently through charge-transfer complexing, electrostatic attractions, and hydrophobic bonding[115]. Adsorption increases as the number of chlorine atoms in the PCB molecule increases[45] and is influenced by the position of the chlorine substituent on the biphenyl ring. The 2-, 4-monochloro isomers and 3,4-dichlorobiphenyl were more likely to bind to organic matter than other biphenyls[69]. This latter finding suggests that electrostatic attractions take place between the chlorine atoms and the humic material.

Chlorinated hydrocarbons such as DDT and dieldrin do not form bound residues in soil as readily as more reactive xenobiotics, such as certain organophosphorus pesticides[70]. This is probably because chlorinated pesticides are relatively inert, and resist chemical and biological conversion to more active compounds[6]. The residues they do form seem to be complexes with humic material. Humus takes up much more pp'-DDT than clay, thus indicating that the organic component of soil is probably responsible for adsorption[94]. Several researchers have shown that humic substances can bind DDT and its analogues[4,26,96]. The content of DDT and related hydrocarbons in marine sediments is correlated with total organic carbon content[28].

Bound DDT has been associated with the lipophilic fraction of organic matter[94]. Apparently this pesticide adsorbs to humus by hydrophobic bonding. Coulombic forces may also play a part in adsorption[93]. Mathur and Morley[80] incorporated methoxychlor, a DDT analogue, into model humic polymers and their results imply that the hydrocarbon binds to soil by forces stronger than those of physical adsorption.

Aliphatic xenobiotics can bind to chemically related components in humus. Khan and Schnitzer[67] isolated dialkyl phthalates from a humic acid by exhaustively extracting it with organic solvents. These authors imply that some of the phthalates may have been xenobiotic. They found that extraction was enhanced for some compounds by subjecting the humic acid to methylation, a method which disrupted the molecular sieve-type structure of soil organic matter. Furthermore, that study indicated that humic material could adsorb more than 2% of its weight in hydrophobic hydrocarbons. Other authors have examined the effects of humic substances on various aliphatic compounds, most notably n-alkanes, fuel oil, dialkyl phthalates and isoprenoid hydrocarbons[9,81].

The binding of ordinarily insoluble hydrocarbons to humus may make them more soluble in water, and this may affect public health. DDT leaches through soil and Ballard[4] assumes that its mobility is due to its association with humic and fulvic acids. Colloidal organic matter serves as a vehicle for DDT in rivers, and fulvic acids make fuel oil more soluble in sea water[9,96]. It is conceivable that, in some cases, humus-hydrocarbon complexes may be potential contaminants of ground water supplies and waterways.

4. *Factors affecting reactions between xenobiotics and humic substances*

The incorporation of xenobiotics into humus depends on many environmental factors which vary with season, climate, soil type, and farming practices. Because of this variability it is not easy to determine whether or to what extent a given xenobiotic will bind to humic material under natural conditions. Therefore, a certain amount of caution must be exercised in interpreting data and extrapolating findings to the environment.

The hydrogen ion concentration affects microbiological, chemical, and autoxidative processes involved in forming bound residues. The phenoloxidases of many soil fungi have pH optima in the range of 4–7[106]. When the pH is above or below these values – as in calcareous or acid soils – the activity of these enzymes could be reduced, and, consequently, they may not be able to form residues.

Table 3. Xenobiotics mentioned in the text and their chemical designation

Common or trade name	Chemical designation
Atrazine	2-Chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine
Benefin	*N*-Butyl-*N*-ethyl-α, α, α-trifluro-2, 6-dinitro-*p*-toluidine
Butralin	4-(1, 1-Dimethylethyl)-*N*-(1-methylpropyl)-2, 6-dinitrobenzeneamine
Carbaryl	1-Naphthyl methylcarbamate
Cypermethrin	α-Cyano-3-phenoxybenzyl(±)Z, *E*-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate
2, 4-D	2, 4-Dichlorophenoxyacetic acid
DCNA	2, 6-Dichloro-4-nitroaniline
DDT	1, 1, 1-Trichloro-2, 2-bis(*p*-chlorophenyl)ethane
Dieldrin	1, 2, 3, 4, 10, 10-Hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-*endo-exo*-5, 8-dimethanonaphthalene
Dinosam	4, 6-Dinitro-*o*-*sec*-amylphenol
Diquat	6, 7-Dihydrodipyrido(1, 2-a: 2′, 1′c)pyrazinediium ion
Diuron	3-(3, 4-Dichlorophenyl)-1, 1-dimethylurea
Fenitrothion	*O, O*-Dimethyl *O*-(4-nitro-*m*-tolyl) phosphorothioate
Flamprop-isopropyl	Isopropyl *N*-benzoyl-*N*-(3-chloro-4-fluorophenyl) alaninate
Fonofos	*O*-Ethyl *S*-phenyl ethylphosphonothiolothionate
Isopropalin	2, 6-Dinitro-*N, N*-dipropyl-cumidine
Linuron	3-(3, 4-Dichlorophenyl)-1-methoxy-1-methylurea
MCPA	4-Chloro-2-methylphenoxyacetic acid
Methoxychlor	1, 1, 1-Trichloro-2, 2-bis(*p*-methoxyphenyl)ethane
Oryzalin	3, 5-Dinitro-N^4, N^4-dipropylsulfanilamide
Oxadiazon	2-*tert*-Butyl-4-(2, 4-dichloro-5-isopropylphenyl)-Δ^2-1, 3, 4-oxadiazolin-5-one
Paraquat	1, 1′-Dimethyl-4, 4′-dipyridylium dichloride
Parathion	*O, O*-Diethyl *O*-(*p*-nitrophenyl) phosphorodithioate
Phosalone	*O, O*-Diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl) methyl phosphorodithioate
Prometone	2-Methoxy-4, 6-bis(isopropylamino)-*s*-triazine
Prometryn	2, 4-bis(isopropylamino)-6-Methylthio-*s*-triazine
Propanil	3, 4-Dichloropropionanilide
TNT	2, 4, 6-Trinitrotoluene
Trifluralin	α, α, α-Trifluoro-2, 6-dinitro-*N, N*-dipropyl-*p*-toluidine

The pH also influences nonbiological bonding by altering the chemical characteristics of the xenobiotic and the humic adsorbent. The charge properties of triazines, for instance, are determined by the hydrogen ion concentration. If protonated, as under acid conditions, triazines can react with humic material. Triazines whose pKa is closest to that of the predominant soil pH are more easily adsorbed to soil organic matter[123]. The behavior of humic substances is also governed by pH. In neutral and alkaline solutions carboxylic and phenolic functional groups on the humic polymer are probably dissociated and xenobiotics may attach to it by cation exchange. In acidic solutions these functional groups are probably not ionized and adsorption occurs by hydrophobic interactions and hydrogen bonding. The latter presumption is supported by a study done by Carter and Suffet[26], who showed that DDT binding to aquatic humus increased as pH decreased. Several researchers[8,61] note that the molecular configuration of humic acid changes with pH, shifting from a stretched formation at neutrality to a coiled one at lower pH. Khan[61] uses this phenomenon as an explanation for differences in the adsorption of paraquat and diquat by humus in neutral and acidic solutions.

The ionic strength of the soil solution is another important environmental factor. Pesticides ionically bound to humic matter may be released with changes in the pH or cation concentration of soil water. The triazine prometone was released from soil organic matter with 0.1 N NaCl[123]. Desorption of paraquat residues from clays have been observed using calcium, aluminum, magnesium, and potassium salts[124]. On the other hand, a high ionic strength apparently enhances the adsorption of hydrophobic compounds. More DDT was bound to organic matter with the addition of Ca^{2+} and with increases in ionic strength[26]. The authors of that study proposed that the reaction of inorganic ions with humic functional groups makes humus more hydrophobic and thus more reactive to hydrophobic chemicals. In seawater an ionic strength of 0.3 appears to be optimal for the complexation of n-alkanes with fulvic acid[9]. The alkanes are incorporated into fulvic micelles, which form only when ionic species are in the solution.

Temperature also has to be taken into account in studying the incorporation of xenobiotics into humus. Incorporation reactions mediated by plant and microbial enzymes are inhibited at high temperatures. In warmer seasons and climates the role of these enzymes in producing bound residues could be reduced.

The effect of temperature on the incorporation of xenobiotics into humus depends upon the process by which the xenobiotic is bound. Increases in temperature will increase binding if the bond formed is strong (e.g., covalent and perhaps hydrophobic and hydrogen bonding). This is apparently the case with atrazine at low pH, which is more strongly adsorbed at 40 °C than at 0.5 °C[76]. On the other hand, Hayes[38] points out that certain weak adsorption processes, such as Van der Waals attractions, are exothermic, and that increases in temperature will decrease xenobiotic binding by humic materials. Cation exchange mechanisms are unaffected by temperature. Burns et al.[17] report that increasing the temperature from 30 °C to 70 °C had no effect on the reaction of paraquat with humic acid.

Moisture levels determine the oxygen content, redox potential, and type of microflora for a soil. The amount of water in soil, then, has a profound effect on the interactions of humus and xenobiotics. When soils are flooded and, therefore, reduced, dinitroanilines and nitrobenzenes are changed to anilines, which can react with humus[37,39,85,98]. In some cases, as with parathion, anaerobic microorganisms appear to be involved in this conversion process[56,89].

The distribution of bound residues in soil organic matter depends upon whether or not a soil is flooded. During an aerobic soil incubation oxadiazon residues were concentrated in the fulvic acid fraction while anaerobic incubation produced an even distribution of residues among all the organic fractions[2]. Residues of ^{14}C-butralin were mostly bound to fulvic acid in aerobic soil and to humin in anaerobic soil[48]. These findings are significant in their implications for pesticide transport in soils and waters. Low molecular weight fulvic acids are more soluble in water than humic acid or humin, and could serve as carriers for xenobiotics.

Irradiation may play a part in the humification of xenobiotics at soil surfaces. The dinitroaniline trifluralin is decomposed to anilines by light[47]. Furthermore, bound prometryn residues apparently can be released from soil organic matter by UV radiation[64]. This finding suggests that certain types of incorporated xenobiotics are not stable, and may later be released when surface organic matter is exposed to sunlight.

Clays may reduce the binding of pollutants by humus. They adsorb pesticides by ion exchange and by weaker physicochemical processes[40]. If soils have an organic matter content lower than 6%, clays may compete with humic materials for binding xenobiotics[111]. Bipyridyliums, as an example, can be removed from humic surfaces by clays[30]. However, in soils with a higher organic matter content, competition is reduced as humus saturates the exchange sites on the clay. It appears that humus-clay complexes are better adsorbents of pesticides than clays alone[60,83]. Pesticides in general seem to preferentially adsorb to organic matter than to clay, but this again depends on the type of pesticide and the kind of clay[19,111].

Particle size and surface area are other influential factors. The adsorption of PCB's by organic materials was inversely related to the adsorbent's particle size and linearly related to the adsorbent's surface area[50]. DDT analogues were associated with humic particles of 8 μm or less in size[28].

Soil amendments also affect xenobiotic incorporation into soil. Lichtenstein et al.[71] found that cow manure, sewage sludge, or atrazine increased bound residues in soils treated with fonofos, an organophosphorus pesticide. This increase could be attributed, in part, to an increase in the amount of organic adsorbents. For parathion, manure and sludge also promoted bound residue formation. Ammonium sulfate, atrazine and the fungicide captafol inhibited the production of inextractable parathion residues. The authors state that the amendments altered the composition of the soil microflora. It has also been found that xenobiotics bound to humus may be mineralized more quickly if soil is treated with chemical analogues of the foreign compound. Aniline, for example, stimulated the mineralization of 3,4-dichloroaniline complexed with humic material[127]. The use of less toxic or more easily degradable analogues in this manner might reduce the persistence of xenobiotics in soil.

5. *Fate of xenobiotic-humus complexes*

The significance and implications of bound xenobiotic residues in soil is not easy to assess. It was the intention of this review to show that the incorporation of man-made chemicals into humus is a complicated process which is influenced by many variables. Therefore, it is a difficult task for scientists and regulatory agencies to establish environmental guidelines which would prevent potential hazards caused by humus-bound xenobiotics.

There is no question that some bound xenobiotic residues persist in soil. Almost 60% of a prometryn treatment was found as bound residues in soil 1 year after application[63]. When dichloroaniline was applied to a German soil, 46% was still in the form of bound residues 2 years after treatment[118]. It is also clear that some of these incorporated soil pollutants can be released later. Plant uptake of released pesticide residues from humus has been documented in a variety of different plant species: oats[34,63], rice[113], mustard[116], and soybeans[49]. Meanwhile, the mechanisms of residue release from soil and organic matter are not known with certainty. Microorganisms appear to be involved to some extent[52,66,79]. Certain soil fungi degrade humic substances, and in so doing can free bound xenobiotics for plant uptake or for further mineralization.

The essential problem is whether or not xenobiotics bound to humus represent a danger to health. At present, most researchers doubt that there are any immediate hazards from bound residues. Turnover time for soil organic matter is quite slow and release of bound residues is probably gradual[42]. Contaminants in plants grown on pesticide treated soil are usually present at microgram per gram concentrations or lower[38,104,113,118]. Still et al.[113] point out that someone would have to eat 10,000 30-g servings of chloroaniline contaminated rice before becoming sick. From this point of view, bound residues do not appear to constitute a real hazard and may even be beneficial as they are, for the most part, inactivated by soil organic matter.

However, the emphasis on the quantity of bound residues in soils and plants is rather misleading if we do not know what these residues are. Most studies done on plant uptake use radiolabeled parent materials which subsequently undergo transformations in soil. The nature and toxicity of these transformation products is largely unknown. In this context it should be noted that some poisons, such as dioxin, are potent at very low concentrations. Even if there are no immediate dangers from bound residues, there is still a possibility that future problems may develop. Over time, xenobiotics released from humus may accumulate in the food chain through bioaccumulation. No one knows what effects the prolonged use of agricultural chemicals will have on soil fertility or public health. It is thus important that we be cautious in evaluating the environmental impact of xenobiotics which are incorporated into soil humus.

More research needs to be done if these complex questions are to be understood and resolved. At present we do not know if humus-bound residues are beneficial or detrimental to man. In the environment both effects may occur under particular conditions. A better understanding of pollutant adsorption by humus may allow us to evaluate and influence the possible fate of these compounds. Irrigation, fertilization, and liming of soil, for instance, could influence the incorporation of xenobiotics into soil organic matter. In the future more controversial methods, such as soil inoculation, enzyme amendments or application of xenobiotic analogues, might serve to reduce soil pollution. However, until more definitive data are obtained, one must be cautious in developing methods which modify the binding of xenobiotics to humus.

Acknowledgment. The preparation of this article was supported in part by the Environmental Protection Agency (EPA; Grant No. R-808165) and by the Pennsylvania Agricultural Experiment Station (Journal Series No. 6661).

1 Aizawa, H., Metabolic maps of pesticides. Academic Press, New York 1982.

2 Ambrosi, D., Kearney, P.C., and Macchia, J.A., Persistence and metabolism of oxadiazon in soils. J. agric. Fd Chem. *25* (1977) 868–872.

3 Ambrosi, D. Kearney, P.C., and Macchia, J.A., Persistence and metabolism of phosalone in soil. J. agric. Fd Chem. *25* (1977) 342–347.

4 Ballard, T.M., Role of humic carrier substances in DDT movement through forest soil. Soil Sci. Soc. Amer. Proc. *35* (1971) 145–147.

5 Bartha, R., Fate of herbicide-derived chloroanilines in soil. J. agric. Fd Chem. *19* (1971) 385–387.

6 Bartha, R., Pesticide residues in humus. ASM News *46* (1980) 356–360.

7 Bartha, R., and Pramer, D., Pesticide transformation to aniline and azo compounds in soil. Science *156* (1967) 1617–1618.

8 Best, J.A., Weber, J.B., and Weed, S.B., Competitive adsorption of diquat^{2+}, paraquat^{2+}, and Ca^{2+} on organic matter and exchange resins. Soil Sci. *114* (1972) 444–450.

9 Boehm, P.D., and Quinn, J.G., Solubilization of hydrocarbons by the dissolved organic matter in sea water. Geochim. cosmochim. Acta *37* (1973) 2459–2477.

10 Bollag, J.-M., Cross-coupling of humus constituents and xenobiotic substances, in: Aquatic and Terrestrial Humic Materials, pp. 127–141. Eds R.F. Christman and E.T. Gjessing. Ann Arbor Sci. Pub., Ann Arbor, Michigan 1983.

11 Bollag, J.-M., Blattman, P., and Laanio, T., Adsorption and transformation of four substituted anilines in soil. J. agric. Fd Chem. *26* (1978) 1302–1306.

12 Bollag, J.-M., and Liu, S.-Y., Degradation of sevin by soil microorganisms. Soil Biol. Biochem. *3* (1971) 337–345.

13 Bollag, J.-M., and Liu, S.-Y., Incorporation of pentachlorophenol into humic acid polymers by a fungal laccase. American Society for Microbiology, Annual Meeting 1983.

14 Bollag, J.-M., Liu, S.-Y., and Minard, R.D., Asymmetric diphenol formation by a fungal laccase. Appl. environ. Microbiol. *38* (1979) 90–92.

15 Bollag, J.-M., Liu, S.-Y., and Minard, R.D., Cross-coupling of phenolic humus constituents and 2,4-dichlorophenol. Soil Sci. Soc. Am. J. *44* (1980) 52–56.

16 Bollag, J.-M., Liu, S.-Y., and Minard, R.D., Enzymatic oligomerization of vanillic acid. Soil Biol. Biochem. *14* (1982) 157–163.

17 Bollag, J.-M., Minard, R.D., and Liu, S.-Y., Cross-linkage between anilines and phenolic humus constituents. Environ. Sci. Technol. *17* (1983) 72–80.

18 Bollag, J.-M., Sjoblad, R.D., and Minard, R.D., Polymerization of phenolic intermediates of pesticides by a fungal enzyme. Experientia *33* (1977) 1564–1566.

19 Bondietti, E., Martin, J.P., and Haider, K., Stabilization of amino sugar units in humic-type polymers. Soil Sci. Soc. Amer. Proc. *36* (1972) 597–602.

20 Bordeleau, L.M., and Bartha, R., Biochemical transformation of herbicide-derived anilines: requirements of molecular configuation. Can. J. Microbiol. *18* (1972) 1873–1882.

21 Bordeleau, L.M., and Bartha, R., Biochemical transformations of herbicide-derived anilines in culture medium and in soil. Can. J. Microbiol. *18* (1972) 1857–1864.

22 Brady, N.C., The nature and properties of soils. 8th edn. MacMillan Publishing Co., New York 1974.

23 Burns, I.G., Hayes, M.H.B., and Stacey, M., Some physicochemical interactions of paraquat with soil organic materials and model compounds. I. Effects of temperature, time and adsorbate degradation on paraquat adsorption. Weed Res. *13* (1973) 67–78.

24 Burns, I.G., Hayes, M.H.B., and Stacey, M., Some physicochemical interactions of paraquat with soil organic materials and model compounds. II. Adsorption and desorption equilibria in aqueous suspensions. Weed Res. *13* (1973) 79–90.

25 Carringer, R.D., Weber, J.B., and Monaco, T.J., Adsorption-desorption of selected pesticides by organic matter and montmorillonite. J. Agric. Fd Chem. *23* (1975) 568–572.

26 Carter, C.W., and Suffet, I.H., Bonding of DDT to humic materials. Environ. Sci. Technol. *16* (1982) 735–740.

27 Chisaka, H., and Kearney, P.C., Metabolism of propanil in soil. J. agric. Fd Chem. *18* (1970) 854–859.

28 Choi, W.W., and Chen, K.Y., Associations of chlorinated hydrocarbons with fine particles and humic substances in nearshore surficial sediments. Environ. Sci. Technol. *10* (1976) 782–786.

29 Cortez, J., and Schnitzer, M., Nucleic acid bases in soil and their association with organic and inorganic soil components. Can. J. Soil Sci. *59* (1979) 277–286.

30 Damanakis, M., Drennan, D.S.H., Fryer, J.D., and Holly, K., The adsorption and mobility of paraquat on different soils and soil constituents. Weed Res. *10* (1970) 264–277.

31 Felbeck, G.T., Jr., Chemical and biological characterization of humic matter, in: Soil Biochemistry, vol. 2, pp. 36–59. Eds A.D. McLaren and J. Skujins. Marcel Dekker, New York 1971.

32 Flaig, W., Beutelspacher, H., and Rietz, E., Chemical composition and physical properties of humic substances. in: Soil Components, vol. 1, pp. 1–211. Ed. J.E. Gieseking. Springer, New York 1975.

33 Führ, F., and Mittelstaedt, W., Plant experiments on the bioavailability of unextracted (carboxyl-^{14}C) methabenzthiazuron residues from soil. J. agric. Fd Chem. *28* (1980) 122–125.

34 Fuhremann, T.W., and Lichtenstein, E.P., Release of soil-bound methyl [^{14}C] parathion residues and their uptake by earthworms and oat plants. J. agric. Fd Chem. *26* (1978) 605–610.

35 Gaillardon, P., Calvet, R., and Gaudry, J.C., Adsorption de quelques phénylurées herbicides par des acides humiques. Weed Res. *20* (1980) 201–204.

36 Golab, T., and Althaus, W.A., Transformation of isopropalin in soil and plants. Weed Sci. *23* (1975) 165–171.

37 Golab, T., Althaus, W.A., and Wooten, H.L., Fate of [^{14}C] trifluralin in soil. J. agric. Fd Chem. *27* (1979) 163–179.

38 Golab, T., Bishop, C.E., Donoho, A.L., Manthey, J.A., and Zornes, L.L., Behavior of ^{14}C oryzalin in soil and plants. Pestic. Biochem. Physiol. *5* (1975) 196–204.

39 Golab, T., Herberg, R.J., Gramlich, J.V., Raun, A.P., and Probst, G.W., Fate of benefin in soils, plants, artificial rumen fluid and the ruminant animal. J. agric. Fd Chem. *18* (1970) 838–844.

40 Green, R.E., Pesticide-clay-water interactions, in: Pesticides in Soil and Water, pp. 3–37. Ed. W.D. Guenzi. American Society of Agronomy, Madison, Wisconsin 1974.

41 Haider, K., Martin, J.P., and Filip, Z., Humus biochemistry, in: Soil Biochemistry, vol. 4, pp. 195–244. Eds E.A. Paul and A.D. McLaren. Marcel Dekker, New York 1975.

42 Hamaker, J.W., and Goring, C.I., Turnover of pesticide residues in soil, in: Bound and Conjugated Pesticide Residues, pp. 219–243. Eds D.D. Kaufman, G.G. Still, G.D. Paulson and S.K. Bandal. Am. Chem. Soc., Washington, D.C. 1976.

43 Hance, R.J., The adsorption of linuron, atrazine, and EPTC by model aliphatic adsorbents and soil organic preparations. Weed Res. *9* (1969) 108–113.

44 Hance, R.J., Complex formation as an adsorption mechanism for linuron and atrazine. Weed Res. *11* (1971) 106–110.

45 Haque, K., and Schmedding, D., Studies on the adsorption of selected polychlorinated biphenyl isomers on several surfaces. J. environ. Sci. Health *B 11* (1976) 129–137.

46 Hayes, M.H.B., Adsorption of triazine herbicides on soil organic matter, including a short review on soil organic matter chemistry. Res. Rev. *31* (1970) 131–174.

47 Helling, C.S., Dinitroaniline herbicides in soils. J. environ. Qual. *5* (1976) 1–15.

48 Helling, C.S., and Krivonak, A.E., Physicochemical characteristics of bound dinitroaniline herbicides in soils. J. agric. Fd Chem. *26* (1978) 1156–1163.

49 Helling, C.S., and Krivonak, A.E., Biological characteristics of bound dinitroaniline herbicides in soils. J. agric. Fd Chem. *26* (1978) 1164–1172.

50 Hiraizumi, Y., Takahashi, M., and Nishimura, H., Adsorption of polychlorinated biphenyl onto sea bed sediment, marine plankton, and other adsorbing agents. Environ. Sci. Technol. *13* (1979) 580–584.

51 Hitchings, E.J., and Roberts, T.R., Degradation of the herbicide flamprop-isopropyl in soil under laboratory conditions. Pestic. Sci. *10* (1979) 1–13.

52 Hsu, T.-S., and Bartha, R., Biodegradation of chloroaniline-humus complexes in soil and in culture solution. Soil Sci. *118* (1974) 213–220.

53 Hsu, T.-S., and Bartha, R., Interaction of pesticide-derived chloroaniline residues with soil organic matter. Soil Sci. *116* (1974) 444–452.

54 Hsu, T.-S., and Bartha, R., Hydrolyzable and nonhydrolyzable 3,4-dichloroaniline humus complexes and their respective rates of biodegradation. J. agric. Fd Chem. *24* (1976) 118–122.

55 Kaplan, D.L., and Kaplan, A.M., Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. Appl. environ. Microbiol. *44* (1982) 757–760.

56 Katan, J., Fuhremann, T.W., and Lichtenstein, E.P., Binding of (^{14}C) parathion in soil: a reassessment of pesticide persistence. Science *193* (1976) 891–894.

57 Katan, J., and Lichtenstein, E.P., Mechanisms of production of soil-bound residues of (^{14}C) parathion by microorganisms. J. agric. Fd. Chem. *25* (1977) 1404–1408.

58 Kaufman, D.D., Bound and conjugated pesticide residues, in: Bound and Conjugated Pesticide Residues, pp. 1–10. Eds D.D. Kaufman, G.G. Still, G.D. Paulson and S.K. Bandall. Am. Chem. Soc., Washington, D.C. 1976.

59 Kazano, H., Kearney, P.C., and Kaufman, D.D., Metabolism of methylcarbamate insecticides in soils. J. agric. Fd Chem. *20* (1972) 975–979.

60 Khan, S.U., Interaction of bipyridylium herbicides with organo-clay complex. J. Soil Sci. *24* (1973) 244–248.

61 Khan, S.U., Adsorption of bipyridylium herbicides by humic acid. J. environ. Qual. *3* (1974) 202–206.

62 Khan, S.U., The interaction of organic matter with pesticides, in: Soil organic matter, pp. 138–171. Eds M. Schnitzer and S.U. Khan. Elsevier Scientific Publishing Co., New York 1978.

63 Khan, S.U., Plant uptake of unextracted (bound) residues from an organic soil treated with prometryn. J. agric. Fd Chem. *28* (1980) 1096–1098.

64 Khan, S.U., Distribution and characteristics of bound residues of prometryn in an organic soil. J. agric. Fd Chem. *30* (1982) 175–179.

65 Khan, S.U., and Hamilton, H.A., Extractable and bound (nonextractable) residues of prometryn and its metabolites in an organic soil. J. agric. Fd Chem. *28* (1980) 126–132.

66 Khan, S.U., and Ivarson, K.C., Microbiological release of unextracted (bound) residues from an organic soil treated with prometryn. J. agric. Fd Chem. *29* (1981) 1301–1303.

67 Khan, S.U., and Schnitzer, M., The retention of hydrophobic organic compounds by humic acid. Geochim. cosmochim. Acta *36* (1972) 745–754.

68 Larson, R.A., and Hufnal, J.M., Jr, Oxidative polymerization of dissolved phenols by soluble and insoluble organic species. Limnol. Oceanogr. *25* (1980) 505–512.

69 Lee, M.C., Griffin, R.A., Miller, M.L., and Chian, E.S.K., Adsorption of water-soluble polychlorinated biphenyl aroclor 1242 and used capacitor fluid by soil materials and coal chars. J. environ. Sci. Health *A 14* (1979) 415–442.

70 Lichtenstein, E.P., Katan, J., and Anderegg, B.N., Binding of 'persistent' and 'nonpersistent' ^{14}C-labeled insecticides in an agricultural soil. J. agric. Fd Chem. *25* (1977) 43–47.

71 Lichtenstein, E.P., Liang, T.T., and Koeppe, M.K., Effects of fertilizers, captafol, and atrazine on the fate and translocation of [^{14}C] fonofos and [^{14}C] parathion in a soil-plant microcosm. J. agric. Fd Chem. *30* (1982) 871–878.

72 Liu, S.-Y., Minard, R.D., and Bollag, J.-M., Coupling reactions of 2,4-dichlorophenol with various anilines. J. agric. Fd Chem. *29* (1981) 253--257.

73 Liu, S.-Y., Minard, R.D., and Bollag, J.-M., Oligomerization of syringic acid, a lignin derivative, by a phenoloxidase. Soil Sci. Soc. Am. J. *45* (1981) 1100–1105.

74 Loll, M.J., and Bollag, J.-M., Protein transformation in soil. Adv. Agron. *36* (1983) 351–382.

75 Loos, M.A., Phenoxyalkanoic acids, in: Degradation of herbicides, pp. 1–49. Eds P.C. Kearney and D.D. Kaufman. Marcel Dekker, New York 1969.

76 McGlamery, M.D., and Slife, F.W., The adsorption and desorption of atrazine as affected by pH, temperature, and concentration. Weeds *14* (1966) 237–239.

77 Martin, J.P., Haider, K., and Wolf, D., Synthesis of phenols and phenolic polymers by *Hendersonula toruloidea* in relation to humic acid formation. Soil Sci. Soc. Amer. Proc. *36* (1972) 311–315.

78 Mathur, S.P., Characterization of soil humus through enzymatic degradation. Soil Sci. *111* (1972) 147–157.

79 Mathur, S.P., and Morley, H.V., A biodegradation approach for investigating pesticide incorporation into soil humus. Soil Sci. *120* (1975) 238–240.

80 Mathur, S.P., and Morley, H.V., Incorporation of methoxychlor-^{14}C in model humic acids prepared from hydroquinone. Bull. environ. Contam. Toxic. *20* (1978) 268–274.

81 Matsuda, K., and Schnitzer, M., Reactions between fulvic acid, a soil humic material, and dialkyl phthalates. Bull. environ. Contam. Toxic. *6* (1973) 200–203.

82 Minard, R.D., Liu, S.-Y., and Bollag, J.-M., Oligomers and quinones from 2,4-dichlorophenol. J. agric. Fd Chem. *29* (1981) 250–253.

83 Mortland, M.M., Pyridinium-montmorillonite complexes with ethyl *N*, *N*-di-*n*-propylthiolcarbamate (EPTC). J. agric. Fd Chem. *16* (1968) 706–707.

84 Mortland, M.M., and Halloran, L.J., Polymerization of aromatic molecules on smectite. Soil Sci. Soc. Am. J. *40* (1976) 367–370.

85 Moza, P., Schneunert, I., Klein, W., and Korte, F., Studies with 2,4′,5-trichlorobiphenyl-^{14}C and 2,2′,4,4′,6-pentachlorobiphenyl-^{14}C in carrots, sugar beets, and soil. J. agric. Fd Chem. *27* (1979) 1120–1124.

86 Mulla, M.S., Mian, L.S., and Kawecki, J.A., Distribution, transport and fate of the insecticides malathion and parathion in the environment. Residue Rev. *81* (1981) 1–172.

87 Murthy, N.B., and Kaufman, D.D., Degradation of pentachloronitrobenzene (PCNB) in anaerobic soils. J. agric. Fd Chem. *26* (1978) 1151–1156.

88 Musso, H., Phenol coupling, in: Oxidative coupling of phenols, pp. 1–94. Eds W.I. Taylor and A.R. Battersby. Marcel Dekker, New York 1967.

89 Parr, J.F., and Smith, S., Degradation of trifluralin under laboratory conditions and soil anaerobiosis. Soil Sci. *115* (1973) 55–63.

90 Parris, G.E., Covalent binding of aromatic amines to humates. 1. Reactions with carbonyls and quinones. Environ. Sci. Technol. *14* (1980) 1099–1106.

91 Parris, G.E., Environmental and metabolic transformations of primary aromatic amines and related compounds. Residue Rev. *76* (1980) 1–30.

92 Peeper, T.F., and Weber, J.B., Activity and persistence of atrazine, procyazine and VEL 5026 as influenced by soil organic matter and clay. Proc. south. Weed Sci. Soc. *29* (1976) 387–398.

93 Pierce, R.H., Olney, C.E., and Felbeck, G.T., Pesticide adsorption in soils and sediments. Environ. Lett. *1* (1971) 157–172.

94 Pierce, R.H., Olney, C.E., and Felbeck, G.T., pp′-DDT adsorption to suspended particulate matter in sea water. Geochim. cosmochim. Acta *38* (1974) 1061–1073.

95 Pillai, P., Helling, C.S., and Dragun, J., Soil-catalyzed oxidation of aniline. Chemosphere *11* (1982) 299–317.

96 Poirrier, M.A., Bordelon, B.R., and Laseter, J.L., Adsorption and concentration of dissolved carbon-14 DDT by coloring colloids in surface waters. Environ. Sci. Technol. *6* (1972) 1033–1035.

97 Pritchard, M.K., and Stobbe, E.H., Persistence and phytotoxicity of dinitroaniline herbicides in Manitoba soils. Can. J. Pl. Sci. *60* (1980) 5–11.

98 Probst, G.W., Golab, T., Herberg, R.J., Holzer, F.J., Parka, S.J., Van der Schans, C., and Tepe, J.B., Fate of trifluralin in soils and plants. J. agric. Fd Chem. *15* (1967) 592–599.

99 Rahman, A., and Matthews, J.L., Effect of soil organic matter on the phytotoxicity of thirteen s-triazene herbicides. Weed Sci. *27* (1979) 158–161.

100 Riley, D., Wilkinson, W., and Tucker, B.V., Biological unavailability of bound paraquat residues in soil, in: Bound and conjugates pesticide residues, ACS Symposium Series *29* (1976) 301–353. Eds D.D. Kaufman, G.G. Still, G.D. Paulson and S.K. Bandal.

101 Roberts, T.R., and Standen, M.E., Further studies of the degradation of the pyrethroid insecticide cypermethrin in soils. Pestic. Sci. *12* (1981) 285–296.

102 Schnitzer, M., Humic substances: chemistry and reactions, in: Soil organic matter, pp. 1–64. Eds M. Schnitzer and S.U. Khan. Elsevier Scientific Publishing Co., New York 1978.

103 Schnitzer, M., and Khan, S.U., Humic substances in the environment. Marcel Dekker Inc., New York 1972.

104 Senesi, N., and Testini, C., Adsorption of some nitrogenated herbicides by soil humic acids. Soil Sci. *130* (1980) 314–320.

105 Sjoblad, R.D., and Bollag, J.-M., Oxidative coupling of aromatic pesticide intermediates by a fungal phenol oxidase. Appl. environ. Microbiol. *33* (1977) 906–910.

106 Sjoblad, R.D., and Bollag, J.-M., Oxidative coupling of aromatic coupounds by enzymes from soil microorganisms, in: Soil Biochemistry, vol. 5, pp. 113–152. Eds E.A. Paul and J.N. Ladd. Marcel Dekker, New York 1981.

107 Sjoblad, R.D., Minard, R.D., and Bollag, J.-M., Polymerization of 1-naphthol and related phenolic compounds by an extracellular fungal enzyme. Pestic. Biochem. Physiol. *6* (1976) 457–463.

108 Spillner, C.J., DeBaun, J.R., and Menn, J.J., Degradation of fenitrothion, in forest soil and effects on forest soil microbes. J. agric. Fd Chem. *27* (1979) 1054-1060.
109 Steelink, C., and Tollin, G., Free radicals in soil, in: Soil Biochemistry, pp. 147-169. Eds A.D. McLaren and G.H. Peterson. Marcel Dekker, New York 1967.
110 Stevenson, F.J., Role and function of humus in soil with emphasis on absorption of herbicides and chelation of microorganisms. Bioscience *22* (1972) 643-650.
111 Stevenson, F.J., Organic matter reactions involving pesticides in soil, in: Bound and conjugated pesticide residues. ACS Symposium Series *29* (1976) 180-207. Eds D.D. Kaufman, G.G. Still, G.D. Paulson and S.K. Bandal.
112 Stevenson, F.J., Humus chemistry: genesis, compoition, reactions. Wiley-Interscience, New York 1982.
113 Still, C.C., Hsu, T.-S., and Bartha, R., Soil-bound 3,4-dichloroaniline: source of contamination in rice grain. Bull. environ. Contam. Toxic. *24* (1980) 550-554.
114 Strek, H.J., and Weber, J.B., Adsorption and reduction in bioactivity of polychlorinated biphenyl (Aroclor 1254) to redroot pigweed by soil organic matter and montmorillonite clay. Soil Sci. Soc. Am. J. *46* (1982) 318-322.
115 Strek, H.J., and Weber, J.B., Behavior of polychlorinated biphenyls (PCBs) in soils and plants. Environ. Pollut. *A 28* (1982) 291-312.
116 Süss, A., and Grampp, B., Die Aufnahme von Adsorbiertem Monolinuron im Boden durch Senfpflanzen. Weed Res. *13* (1973) 254-266.
117 Van Alfen, N.K., and Kosuge, T., Metabolism of the fungicide 2,6-dichloro-4-nitroaniline in soil. J. agric Fd Chem. *24* (1976) 584-588.
118 Viswanathan, R., Scheunert, I., Kohli, J., Klein, W., and Korte, F., Long-term studies on the fate of 3,4-dichloroaniline-^{14}C in a plant-soil system under outdoor conditions. J. environ. Sci. Health *B 13* (1978) 243-259.
119 Waksman, S.A., and Iyer, K.R.N., Contribution to our knowledge of the chemical nature and origin of humus: I. On the synthesis of the 'humus nucleus'. Soil Sci. *34* (1932) 43-69.
120 Wang, T.S.C., and Li, S.W., Clay minerals as heterogeneous catalysts in preparation of model humic substances. Z. Pfl Ernähr. Düng. Bodenk. *140* (1977) 669-676.
121 Wang, T.S.C., Li, S.W., and Ferng, Y.L., Catalytic polymerization of phenolic compounds by clay minerals. Soil Sci. *126* (1978) 15-21.
122 Weber, J.B., Adsorption of buthidazole, VEL 3510, tebuthiuron, and fluridone by organic matter, montmorillonite clay, exchange resins, and a sandy loam soil. Weed Sci. *28* (1980) 478-483.
123 Weber, J.B., Weed, S.B., and Ward, T.M., Adsorption of *s*-triazines by soil organic matter. Weed Sci. *17* (1969) 417-421.
124 Weed, S.B., and Weber, J.B., The effect of cation exchange capacity on the retention of diquat and paraquat by three-layer type clay minerals. I. Adsorption and release. Soil Sci. Soc. Am. Proc. *33* (1969) 379-382.
125 Wheeler, W.B., Stratton, G.D., Twilley, R.R., Ou, L.-T., Carlson, D.A., and Davidson, J.M., Trifluralin degradation and binding in soil. J. agric. Fd Chem. *27* (1979) 702-706.
126 Wolf, D.C., and Martin, J.P., 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. Am. Proc. *40* (1976) 700-704.
127 You, I.-S., and Bartha, R., Stimulation of 3,4-dichloroaniline mineralization by aniline. Appl. environ. Microbiol. *44* (1982) 678-681.
128 You, I.-S., Jones, R.A., and Bartha, R., Evaluation of a chemically defined model for the attachment of 3,4-dichloroaniline to humus. Bull. environ. Contam. Toxic. *29* (1982) 476-482.

0014-4754/83/111221-11$1.50+0.20/0
©Birkhäuser Verlag Basel, 1983

Use of specialized microbial strains in the treatment of industrial waste and in soil decontamination

by R.K. Finn

School of Chemical Engineering, Olin Hall, Cornell University, Ithaca (New York 14853, USA)

A natural tendency among most waste treatment specialists is to regard any use of tailor-made cultures as useless in the effort to abate pollution. Such skepticism is deeply founded and is expressed, for example, by engineers whose training and experience lie not so much in microbiology as in the workaday world of processing enormous quantities of dilute municipal sewage containing traces of every imaginable substance. Traditionally, and to some extent even today, the practice has been to dilute the organic matter and then to disperse it broadly into the environment. Ecologists, by virtue of to their training, are usually primarily concerned with observing and sampling natural environments. Again, the systems they study are complex and disparate. The general criterion for assessing the stability and 'health' of any microcosm has to do with the diversity of species - both flora and fauna, including a broad array of microorganisms - of which that microcosm is comprised. Consequently, one hears such objection as, 'Your cannot run a waste-treating facility like an antibiotics plant' or 'Any open system will seek its own equilibrium and will arrive at the same steady state no matter what the initial conditions imposed from outside', or 'Monocultures are unstable'.

There is a truth in such skepticism. One cannot afford to be sanguine in the face of many failures by overzealous advocates of a controlled environment. One of the earliest proponents was Charles Darwin himself. Having established the beneficial effects of common earthworms on soil structure and fertility, he pictured their widespread use as inoculants in poor soils. His suggestions did not work in practice. Earthworms are indeed abundant in nature and when conditions are favorable they will multiply rapidly, but in poor soils they will die out. In more recent times there have been similarly unsuccessful attempts to establish free-living *Azotobacter* in soils so as to fix atmospheric