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Formation of non-bioavailable organic residues in soil: Perspectives for site remediation

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Abstract

Whenever possible, total clean-up of soils and sediments should have priority over methods to contain the pollutants in the soil environment in a way which reduces their potential eco-toxicological effects. Nevertheless, often a very important fraction of the pollutant remains non-available to the cleaning process, either physico-chemical or biological. This constitutes a major obstacle for both environmental technologists and legislators. Yet, the concept of non-extractable organic residues is well accepted in the EU-legislation for pesticides. In this context, an assessment is made to bind organic pollutants to soil. Physical sorption (comprising surface adsorption, absorption and migration in micro- and nanopores) and chemical binding are examined in terms of quantities and kinetics. Chemical binding offers at present no direct possibilities for practice. Making toxic pollutants less bioavailable by increasing physical sorption represents a pragmatic approach to contractors and regulators. For organic pollutants with acceptable concentration in the soil solution of the order of 1 mg/l, a sorptive loading of the order of 10 000 mg pollutant per kg activated carbon respectively organic matter appears a workable assumption. In case of toxic substances such as pesticides which have a 1000 times lower acceptable level, a sorptive loading of up to 10 mg organic pollutant per kg sorbent can be used. Non-bioavailable pollutants can be considered as representing no direct harm to the environment. In practice, the application of up to 100–200 kg dry weight quality compost per ton dry weight soil or alternatively the supplementation of other sorbents such as powdered activated carbon (up to 100 kg per ton soil) offer possibilities to cost-effective remediation of organic pollutants. Yet, aspects of worst-case ecotoxicology as e.g. excessive leaching with dissolved humic substances or ingestion of soil containing substantial amounts of poorly extractable contaminants by human and soil organisms, need to be examined.

Introduction

The increasing concern about soil quality has generated a genuine interest in techniques to remove pollutants from soil. In case this can be achieved, total removal has to be the method of choice. Remediation methods such as venting, solvent flushing, leaching, washing, thermal desorption, . . . have as target to remove as much pollutant as possible in a minimum of time (Blackburn & Hafner 1993; Troxler et al. 1993; Augustijn et al. 1994; McAdams 1994). Methods which make use of microbial degradation, either natural or augmented, e.g. by genetic manipulation, aim at the removal of the pollutant through conversion to CO_2 and other mineral components. Nowadays, the most successful applications of bioremediation for site clean-up have been those that stimulate the activity of naturally occurring microorganisms by the addition of nitrogen, phosphorus and potassium fertilizers, with adequate aeration (Genouw et al. 1994; Zhou & Crawford 1995).

In many cases, an important fraction of pollutant remains untouched by the cleaning process, either physico-chemical or biological. The amount of pollutant remaining in the soil constitutes in the field of soil depollution a point of major concern and debate in relation to risk assessment. In the field of soil pesticide chemistry however, pragmatism has resulted in the fact

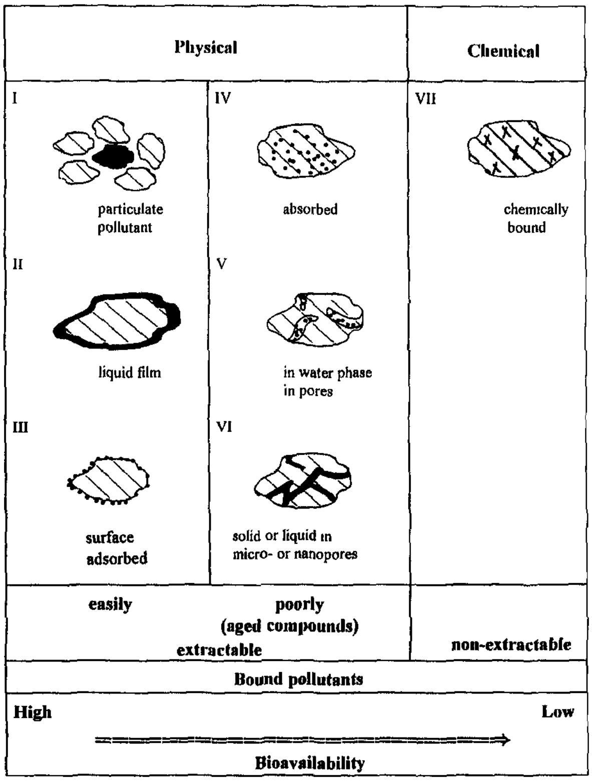


Figure 1. Different physical and chemical forms of organic pollutants in soil; I: solid particles; II: liquid film; III: adsorbed onto soil; IV: absorbed into soil; V: in the waterphase of soil pores; VI: as a separate phase in soil pores; VII: chemically bound to soil (adapted from Volkering 1996).

Table 1. Examples of bound pesticide residues in soils (after Calderbank 1989)

Structural type	Bound residues (% of applied)	Parent detected[a]
Herbidices		
Anilides and urea	34–90	No
Bipyridyliums	10–90	Yes
Nitroanilines	7–85	No
Phenoxy acids	28	No
Phosphonates (glyphosate)	12–95	Yes
Triazines	47–57	Yes
Insecticides		
Carbamates	32–70	Yes
Organochlorines	7–25	?
Organophosphates	18–80	Yes
Pyrethroids	3–23	No
Fungicides		
Chlorophenols	45–90	?
Nitroaromatic (dinocap)	60–90	?

[a] Indicates where parent was positively identified. In cases 'No' or question mark, the method of extraction may have decomposed parent molecule.

that the legislator has accepted that many pesticides do not get completely degraded, but become 'bound' to the soil. For a large number of pesticides, this binding process is the principal cause of their 'dissipation' (European Union 1994). Yet, it must be emphasized that in most cases, it is not the parent compound that gives rise to bound residues. Actually, for many pesticides, the binding is a consequence of transformation of the parent product into more reactive species such as phenolic and amino derivatives.

Figure 1 illustrates the different forms the organic pollutant can have in soil. To minimize confusion, a pragmatic approach based on chemical extraction procedures is proposed. The term easily extractable relates to the fraction which is set free rapidly (< 1 h of contact) at room temperature (Mahro et al. 1994; Hatzinger & Alexander 1995; Vanneck 1996). The term 'poorly extractable' relates to pollutants which either by ad- or absorption, diffusion in nanopores, ... are hardly or not all extractable by normal solvents. However, by extensive refluxing with a solvent (8–24 h) at higher temperatures (e.g. 40 °C for dichloormethaan, 34 °C for diethylether) in a Soxhlet apparatus (Dibble & Bartha 1979; Genouw et al. 1994; Hatzinger & Alexander 1995; Vanneck et al. 1996), these 'poorly extractable' pollutants can be set free. Alexander (1995) stated that aged compounds can be extracted by some organic solvents, often under vigorous conditions. The term aging does not include reactions that alter the structure of the molecule, for example polymerization or covalent binding to humic substances (Hatzinger & Alexander 1995). Hence, aged compounds are considered to belong to the category of poorly extractables. The term 'non-extractable' relates to altered structures which can only be set free upon chemical modifications of the molecules concerned. As schematized in Figure 1, the term 'bound residues' is proposed to relate to all residues retained in a physical or chemical way in the soil, reversible or irreversible. There is no sharp borderline which compounds are available to biology, but it is considered that the probability of bioavailability decreases from the left of the figure to the right.

In this context, an assessment of the potential to bind organic pollutants to soil and to consider bound pollutants with the same pragmatism as is applied to bound pesticides, is worthwhile. This paper reviews how bound residues are formed in the soil with special emphasis to the possible perspectives of these processes for soil depollution in practice.

Legal aspects

During the last decades, many reports have demonstrated that bound pesticide residues in soil are formed with all classes of pesticides so far investigated. Table 1 (after Calderbank 1989) indicates that some 20 to 70% of the amounts of active substances of pesticide molecules applied in the field, become bound to the soil. Bound residues are formed with the parent compound product or with its degradation products. Yet, the information presented in Table 1 is of limited utility without more information on the contact time associated with the bound residues reported, the soil types, and the method(s) used to assess the percentage that is bound.

The binding of pollutants to soil humus has two important consequences. First, the amount of available compounds to interact with the biota is decreased. Secondly, products are frequently made insoluble which results in a reduction on the movement of chemicals by leaching, thus preventing the contamination of aquatic environments.

As a rule of thumb, an average amount of 50% of bound residues can be proposed for pesticides under normal agricultural practices. The definition of 'non-extractable residue' has been a subject of discussion for a long time (Khan 1982). In the current paper, the term is considered in the following context: chemical species originating from the original chemical applied, that cannot be extracted from the soil by methods which do not significantly change the chemical nature of the organic chemicals. This definition parallels the one the European Union uses in terms of crop protection chemicals (European Union 1994). The European Union (1994) considers those fragments of the original molecule 'which through metabolic pathways are leading to natural products' not to be part of the non-extractable residue.

However, since this is a quantity difficult to estimate, we like to limit the definition of natural products to microbial biomass. Unlike 'natural products', microbial biomass is a well-defined fraction which can be analyzed, either by fumigation incubation or by fumigation extraction methods (Vance et al. 1987). Hence, our definition of non-extractable residues comprises those non-biomass residues which contain moieties from the originally applied chemical in a form which is not readily set free from the soil matrix. Indeed, to totally recover these moieties, destructive methods such as strong acids, alkali or incineration are required.

Table 2. Physical properties of soil colloids and of powdered active carbon as a reference (after Calderbank 1989)

Soil constituent	Cation exchange capacity (meq/100 g)	Surface area (m^2/g)
Organic matter	200–400	500–800
Vermiculite	100–150	600–800
Montmorillonite	80–150	50–150
Dioctahedral vermiculite	10–150	50–800
Illite	10–40	65–100
Chlorite	10–40	25–40
Kaolinite	3–15	7–30
Oxides and hydroxides	2–6	100–800
Powdered active carbon	–	1000

In the legislation of the European Union (1994) pesticides are subjected to strict requirements in terms of disappearance time, leaching in the groundwater etc. To prevent unlimited accumulation of the pesticide, the quantity which disappears as such and reappears (e.g. via ^{14}C-tracing) as non-extractable residue, must not rise above 70% of the parent compound applied within 100 days after application. Obviously, this end point relative to pesticide molecules can not be considered absolute but it can serve as a reference for evaluating the fate of other pollutants.

The mechanisms of soil binding

Sorption

The term adsorption relates to molecules which are adsorbed on or in the soil matrix without the formation of covalent bonds. For many soil scientists, surface sorption is considered as the quite rapid and reversible partition process of the chemical between water and solid phase. This sorption is strongly dependent on the hydrophobic/hydrophilic properties of the chemical as well as on its concentration in the water phase (Freundlich model). Part of this adsorption is due to the minerals, especially the clay fraction, and part to the organic matter (Table 2). Whereas clay minerals are responsible for adsorption of polar and hydrophilic compounds, organic matter consists of hydrophilic and

Table 3. Data on surface adsorption of organic pollutants to different matrices

Chemical	Matrix	mg sorbed per kg matrix dry matter	Reference
2,4-D	Compost	10	Michel et al. 1995
Chlorothalonil	Farmyard manure	50	Katayama et al. 1995
Norflurazon	Activated carbon	2–20	Lamoreaux et al. 1989
TCE	Peat moss	1800	Zytner 1992
PCP	Anaerobic sludge	440–770	Kennedy and Pham 1995

Table 4. Butanol extraction (< 1 h) of chemicals from soils after aging under sterile conditions

	Aging time (days)	% Extracted
Phenanthrene added 10 mg/kg		
Lima loam (4% org. matter)	0	99
	13	96
	27	89
	84	87
Edwards muck (19.3% org. matter)	0	95
	13	67
	27	64
	84	61
4-Nitrophenol added 10 mg/kg		
Lima loam	0	82
	40	62
	103	56
Edwards muck	0	75
	40	49
	103	45

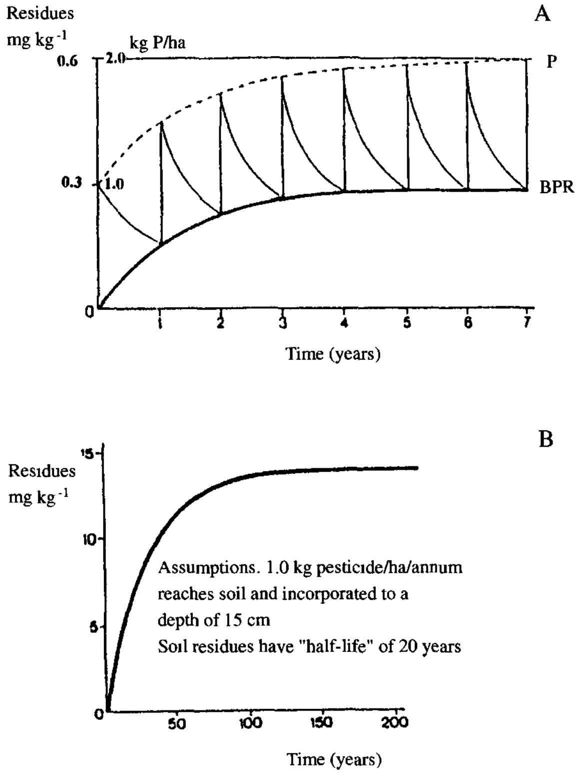
(after Hatzinger & Alexander 1995)

hydrophobic regions, which results in an adsorption capability for both polar or charged and apolar or lipophilic chemicals. With respect to surface sorption in soil, it is most important since both its CEC as well as its surface area are higher than any other soil component (Table 2). Table 3 summarizes some other data on surface adsorption of pollutants to soil. For a large number of pesticides, dosed at 0.70 to 74 mg kg^{-1}, with soil organic matter contents ranging from 1 to 85%, the amount of bound residues formed ranged from 17 to 685 mg kg^{-1} organic matter. The average was 108 ± 29 mg kg^{-1} organic matter (Khan 1982).

Hatzinger and Alexander (1995) describe the effect of aging of phenanthrene and 4-nitrophenol in soils with different organic matter content (ranging from 2.3% to 19.3%). The amount of residue extracted by butanol was followed with time for sterile incubated samples (Table 4). Significant occurrence of bound residues (indicated by sorption beyond surface adsorption) required a period of several weeks to months. Aging is most probably a matter of adsorption in micro- or nanopores and maybe of absorption in the soil matrix. For doses of 10 mg chemical per kg soil, a maximum of about 4.4 mg of 'poorly or non-extractable' residues was noticed with 4-nitrophenol for the soil with 4% organic matter. Hence, Hatzinger and Alexander (1995) found some 100 mg of bound residue per kg soil organic matter. In some cases, possibly with specific pollutants, up to about 1000 mg of organic pollutants can be bound per kg of soil organic matter (Khan 1982). The overall message from these considerations on sorption of organic pollutants is that, calculated on the amount of organic matter present in soil, an order of magnitude of 10 mg of organic molecule sorbed per kg soil organic matter can be used and represent a safe loading under a wide spectrum of conditions.

It must be emphasized that the residues adsorbed to soil organic matter are subject to microbial degradation. It is indeed reasonable to assume, based on current evidence, that the adsorbed parent compound or metabolite is in equilibrium in trace amounts with the soil solution and thus available for degradation. Moreover, it has not been excluded so far that microorganisms might be capable to directly act on the surface-sorbed compounds. Microbial uptake of such surface-bound substrates stimulates the desorption by maximising the concentration gradient which exists over very short distances between the particle surface and the cell wall. This 'driven' desorption can even be more pronounced if microbial cells are attached to the surface or more generally if bacteria are at the same place, at the same time, as the sorbed substrate (Rijnaarts et al. 1990; Guerin & Boyd 1992; Harms & Zehnder 1995).

Figure 2 demonstrates that the concentration of bound residues is considered to level off, depending of course on their molecular degradation rate and their application dose. The examples refer to relative recal-



Assumptions. 1.0 kg pesticide/ha/annum reaches soil and incorporated to a depth of 15 cm
Soil residues have "half-life" of 20 years

Figure 2. Theoretical plateau levels for bound pesticide residues (BPR). (A) For annual applications of 1 kg pesticide (P) and first order half-life of 1 yr for both P and BPR (the assumption is made that 1 ha = 3 × 10^6 kg). (B) After repeated annual applications of 1 kg/ha and a half life of 20 yr (after Calderbank 1989).

citrant chemicals (first order half-lives of 1 and 20 year respectively) but to relatively low application rates (1 kg pollutant per ha). The message for practice is that in soil pesticide ecotoxicology, bound pesticide residues in the order of 0.5–20 mg organic compound per kg soil, are at present occurring and not in disagreement with general environmental policy. In other words, if one assumes agricultural soils at an average organic matter content of 2%, it appears from these data that from 25 to 1000 mg organic pesticide residues can be bound per kg organic matter without imposing problems with the levels of pesticides in the groundwater; that is not giving rise to concentrations above 1 μg/l. Yet, it is worthwhile that the level of risk related to the apparant reference level of 100 mg of organic pesticide residue sorbed per kg soil organic matter is subjected to regular update and review.

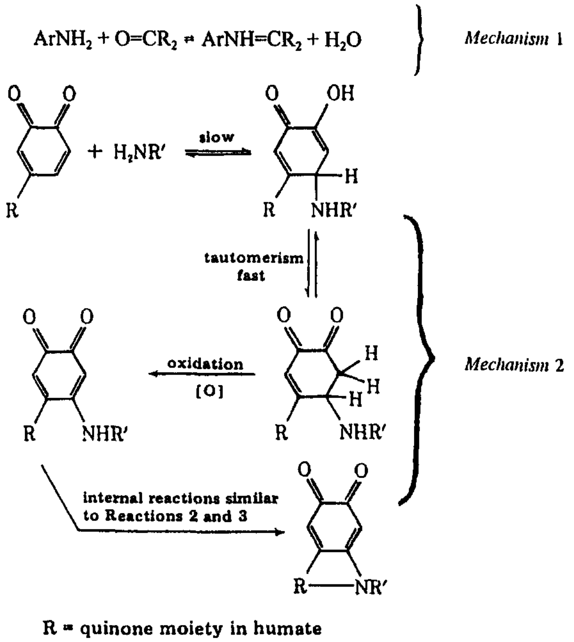


Figure 3. Reversible and irreversible binding of amines to carbonyl groups of soil humates. R = quinone moiety in humate (after Parris 1980).

Chemical binding

Chemical binding is the result of a covalent bond that is formed between the pollutant and the humic matter of the soil. A stable chemical linkage is formed and this reaction is known as 'Oxidative Coupling'. The formed complexes are highly resistant to acid or base hydrolysis, thermal treatment and microbial degradation.

Aromatic amines are perhaps the most susceptible group of aromatic pollutants for coupling reactions. Parris (1980) gave a detailed analysis of the covalent binding of aromatic amines to humate (Figure 3). The primary amines may bind to humate in two ways. A reversible equilibrium is established, which may represent formation of imine linkages with the humate carbonyls. There is also a slow reaction possible that is not readily reversed. The reaction is thought to represent addition to quinone rings (present in humic matter due to oxidation of phenols), followed by tautomerization and oxidation to give an amino-substituted quinone. The amino group may react further by the same sequence to form a variety of nitrogen heterocyclic compounds. Studies by Tatsumi et al. (1994) have further established these reaction mechanisms. The amounts of aromatic amines thus bound to humates range in the order of 500 mg per kg humate organic matter.

Microbial enzymes, inorganic chemicals and clay minerals can catalyse these oxidative reactions. Different enzymes have been tested (Bollag 1992; Tatsumi et al. 1994). Shannon and Bartha (1988) demonstrated that many but not all phenolic pollutants could be immobilized to the soil organic matter by use of the horseradish peroxidase-H_2O_2 system. They could bind up to 100 mg pollutants, such as 2,4-dichlorophenol, per kg soil dry weight, corresponding to a binding of about 10 000 mg of pollutant per kg soil organic matter.

A large amount of research efforts have been directed towards the use of polyphenol oxidases. These enzymes require bimolecular oxygen and no cofactors. They are subdivided into two classes. The first group are the tyrosinases which form o-diphenol from the parent compound and subsequently release a highly reactive o-quinone. The latter can then slowly further polymerise spontaneously at high pH. Laccases, the second subgroup, transform phenolic compounds into anionic free radicals. The latter are very reactive and polymerise further very rapidly. Bollag (1992) did experiments with phenols containing one to five chlorines which were cross-coupled with syringic acid by the laccase from *Rhizoctonia praticola*. He observed and quantified (m/z value of molecular ion) the presence of various phenolic oligomers (dimer, trimer, tetramer and pentamer) in all the cases. High molecular weight oligomers were mainly formed and the tendency to polymerise increased as the number of halogenated atoms present in the molecule increased.

At present, studies indicate that residues which are chemically bound to the soil humates are toxicologically inactive (Calderbank 1989). Even chloro-humus polymers were found to be non-toxic (Field et al. 1995). Moreover, there is no history of toxic remobilization. Nevertheless, although the humus polymers are ecotoxicologically harmless, special attention is needed with respect to intermediary products. Indeed, specially when using enzymes to induce cross-coupling reactions, several intermediates more toxic than the former (parent) compounds may be formed (i.e. dioxins). So toxicity aspects must be dealt with carefully and each case must be studied separately (Svenson et al. 1989; Öberg et al. 1990).

Finally, these chemically bound residues are also susceptible to slow mineralization when they are released (Saxena & Bartha 1983; Dec & Bollag 1988; Calderbank 1989), be it at the rate of normal soil humus, i.e. 1–2% conversion to CO_2 per year.

Overall quantities

Considering the two mechanisms by which pollutants can be bound to the soil, it appears that physical sorption (in its different forms) is of the order of 1 mg per kg soil while chemical binding is of the order of 10 mg per kg soil. Expressed per kg organic matter, the values for physical sorption and chemical binding are of the order of 100 and 1000 mg/kg organic matter respectively, which is considerable. In this context, it should be indicated however that physical sorption seems to be a general phenomenon for many pollutants, while chemical binding appears to be more restricted to certain molecules. In case large amounts of recalcitrant molecules are present, it is of interest to examine to what extent these two mechanisms of soil binding can be of technological interest and environmentally acceptable.

It is generally assumed that biodegradation of non-dissolved pollutants is minimal. Yet, Figure 2 illustrates that in pesticide chemistry, bound residues are considered to be mineralised. Enkiri et al. (1995) demonstrated that biphenyl adsorbed to hydrophobic polyurethane foam degraded twice as fast as in water. Hence, this area of biodegradation of the bound pollutants needs to be documented particularly in terms of the influence of stacking phenomena of molecules at surfaces and possible alterations in biodegradation patterns and rates.

A major concern in relation to this binding of pollutants relates to worst-case ecotoxicological scenarios of soil disturbance and particularly soil ingestion. Considering the case of soil disturbance, it is conceivable that the soil becomes flooded with water containing hydrophobic dissolved humic substances. The latter can reduce, through binding of organic contaminants, the sorbability and facilitate the desorption of pollutants sorbed to the solid phase humic matter (Rebhun et al. 1996) (Figure 4). Similarly, Janzen et al. (1996) reported that the application of compost extract at a concentration of 400 mg organic matter per 1 can enhance the desorption of hydrophobic substances such as α-naphtol and naphthalene from soil (the Koc decreased in the range of 5–25%). Cases like this, as well as the application of detergents, thus might set free pulses of adsorbed pollutants but it certainly is clear that these pulses are only relating to a fraction of the bound chemicals. Moreover, it remains quite possible that the existing microbiota in such instances might be capable to mineralize the newly bioavailable organics. Another case is the uptake of soil into the animal body.

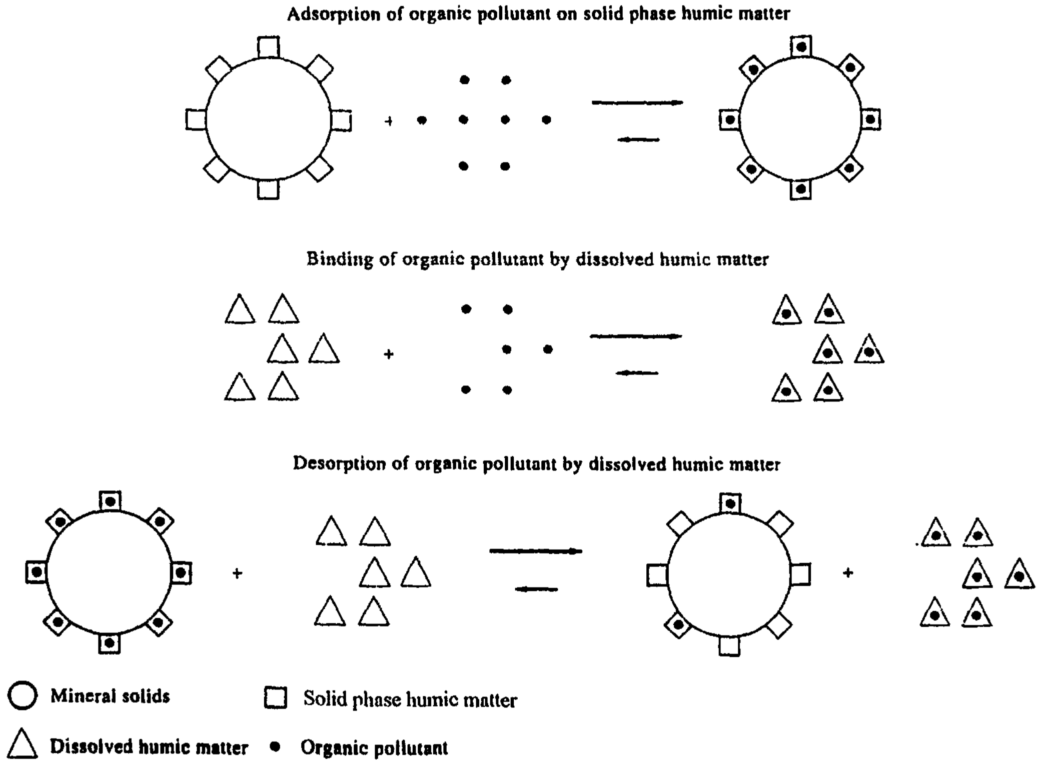


Figure 4. Modes of interaction of organic contaminants with solid material and dissolved humic substances (after Rebhun et al. 1996).

It is possible that people or animals might take up soil in their digestion tract. The strongly acidic conditions in the stomach, the highly surfactant action of bile salts in the small intestine and finally the intensive fermentation in the large intestine might potentially release some of the bound molecules. In this respect, research by means of simulator systems of the gastro-intestinal systems might offer an affordable approach to acquire appropriate information (Molly et al. 1993, 1994). To verify the non-ecotoxicity of the bound pollutants on soil organisms, standard earthworm toxicity tests also might be set up.

Kinetics of binding

It is not only important to have an estimate of how much of an organic pollutant can be bound to the soil or to its organic matter fraction, it is also very important to have an estimate of the reaction rates involved.

Surface adsorption

Hatzinger and Alexander (1995) monitored the butanol extraction of phenanthrene and 4-nitrophenol in sterile soil samples and found that it decreased mainly in the first 3 months. The biological availability however continued to decrease with time, even over a period of a year.

Pignatello and Huang (1991) studied the reversible sorption of atrazine and metolachlor residues in field soil samples. They measured the ‘equilibrium’ linear sorption constant K_d in the soil in the traditional manner by constructing sorption isotherms with freshly added herbicide and taking into account a period of 24 hours of incubation. Moreover, they measured the K_{app}, which is the constant related to the total sorbed concentration.

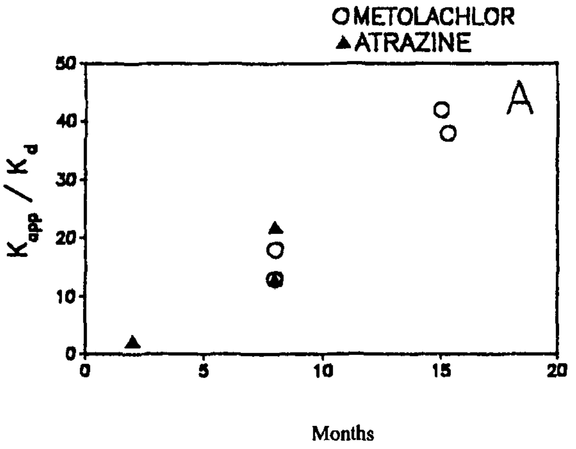


Figure 5. Effect of residue age on K_{app}/K_d for 2 pesticides (after Pignatello & Huang 1991).

Consider the linear Freundlich partitioning model:

$$S = K_d \cdot C$$

with

S = amount of pesticide sorbed (mg/kg)
C = the measured solution concentration (mg/cm^3)
K_d = linear sorption constant (cm^3/kg)

The total sorbed concentration S_T, is the sum of the labile fraction and the resistant fraction. The former partitions reversibly between solid and solution according to the sorption parameter K_d, whereas the latter remains wholly associated with solids during the incubation period. S_T = S sorbed in a labile reversible way + S sorbed in a more permanent way = $K_{app} \cdot C$.

For the pesticides studied, the K_d was of the order of 1 to 2 for different soil types. However, the K_{app} was of the order 30 to 40 (Figure 5). This means that with time, some 30–40 times more chemical is sorbed to the soil matrix. The most important message from this paper was that the ratio of K_{app}/K_d continues to rise with time and requires some 20 months to reach the values of 30–40. This rise clearly corresponds with the so-called aging of the chemical due to continued diffusion of labile chemical into more remote or 'stronger' binding sites in the soil.

Apparently, it takes a long time to bind pesticides to such extent that the concentration in the solution is a factor 30–40 lower than that present in the total soil matrix. Yet, Pignatello and Huang (1991) demonstrated that for native herbicides in soil, concentrations in solution after 24 hours of incubation in water were of the order of 1 μg/l while the total amount was in the range of 30–40 μg/kg dry weight of soil. The main question which arises in this context is how one can possibly accelerate the diffusion of the chemical to the remote sorption sites.

A rather remarkable consequence of this aspect is that soils, which are cleaned, can later on appear to be polluted again. Figure 6 illustrates the difference in the process of bioremediation in freshly and 'aged' contaminated soil. As it can be observed in the figure, in freshly contaminated soil, contamination only reaches macropores and the outer, relatively easy accessible regions of aggregates (Figure 6A). After bioremediation, the pollutant is completely depleted in the macropores, while a small residue remains in the outer regions of the aggregates (Figure 6B). Opposed to this, 'aging' contaminations have penetrated the aggregates completely, including regions with extreme narrow and tortuous micropores (Figure 6C). After bioremediation macropores and the outer regions of the aggregates are clean, but a considerable residual amount of contaminant remains in the internal part of the aggregates (Figure 6D). As a result of the bioremediation process, steep diffusion gradient exists between the inner part of the aggregates and the macropores which may result in a very slow diffusion to the macropores again. This explains why sites which are supposed to be cleaned by *in-situ* bioremediation, sometimes appear to be polluted again after a few years (Bosma 1994).

In water treatment with activated carbon, adsorption kinetics are also far from clear. The phase equilibrium between fluid and sorbent is not spontaneously established, as the solute molecules first have to be transferred through the film that surrounds the adsorbent before they diffuse through the pores and are taken up by the active surface. Particle size and pore structure are the major influencing parameters for the kinetic properties of activated carbon. The smaller the particle size, the shorter are the distances to be covered by the solute from the liquid phase to the adsorption site and the faster the adsorption proceeds. Accordingly, the adsorption equilibrium is attained fastest with powdered activated carbons. With granular carbons as commonly employed in fixed bed adsorbers, the filtration rate in the adsorber and the pore structure of the activated carbon have a great influence on adsorption kinetics. As it has not been possible so far to establish a correlation between the pore structure and the diffusion parameters, the adsorption kinetics have to be experimentally determined for each individual case. Yet, generally contact times of the order of hours suffice.

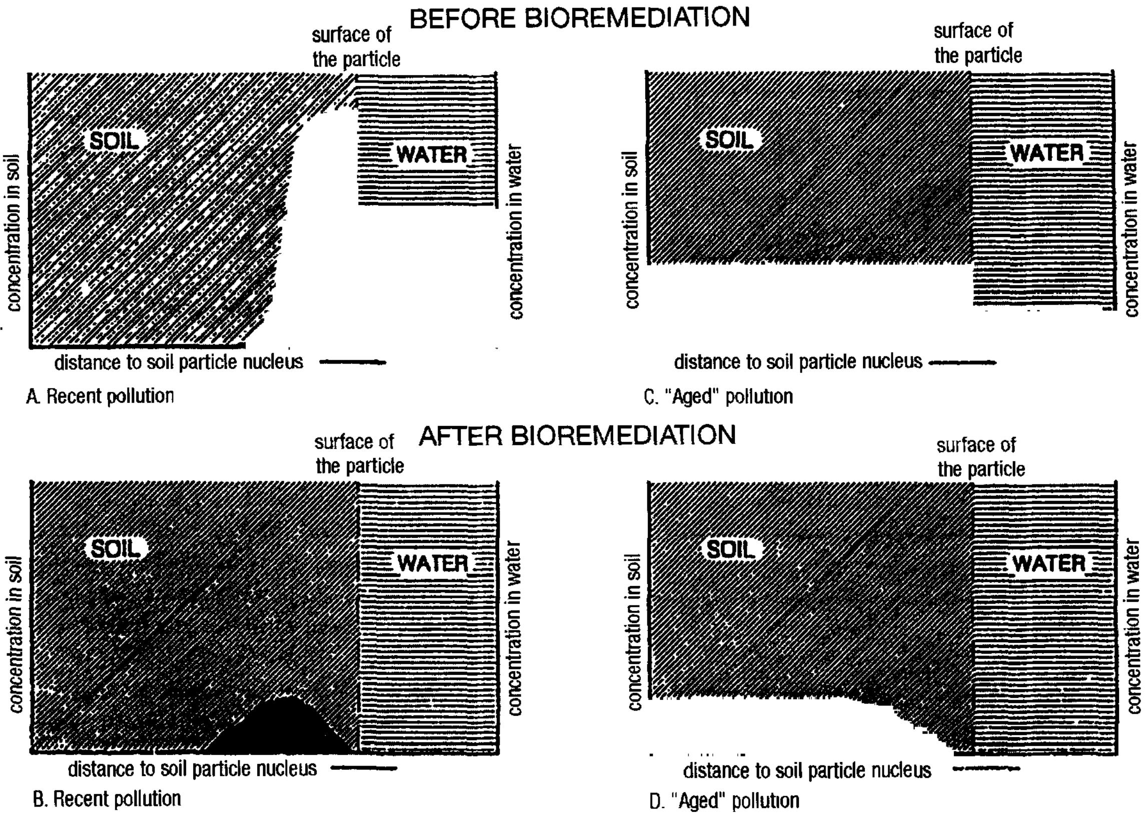


Figure 6. Schematic visualisation of the distribution of a pollutant in a soil particle and surrounding liquid. The vertical scales are different in a way that the soil concentration >> water concentration (after Bosma 1994).

Chemical binding

The work of Tatsumi et al. (1994) on the enzymatic coupling of chloroanilines with phenolic acids demonstrates that these reactions can occur to completion in a matter of hours (Figure 7). Shannon and Bartha (1988) immobilized phenolic pollutants using an extracellular laccase during an incubation period of 3 to 4 weeks. These results and other different investigations suggest that it is necessary to study each particular pollutant and the enzyme used under the most adequate conditions of temperature, pH, reactant availability, etc. to maximize reaction rates. Biochemical binding can obviously be subject to biotechnological optimization in case this might be considered a worthwhile practical approach.

Overall rates of binding

The considerations mentioned above indicate that the chemical binding is certainly the fastest process specially if it is brought about by the proper enzymes under the right conditions. Secondly, in terms of rate comes the reversible surface adsorption; this can take days to weeks. Substantial migration into small pores might take months. Diffusion phenomena become less important when soil aggregates or particles are small sized, because such aggregates are characterised by short diffusion pathlengths and high (external) surface area (Rijnaarts et al. 1990; Scow & Hutson 1992).

Strategies for binding pollutants

Considering the capacity of the different mechanisms to bind xenobiotics to humus or other matrices as reviewed above, as well as their respective kinetics, it is of interest to evaluate which of these mechanisms provides an effective biotechnological potential.

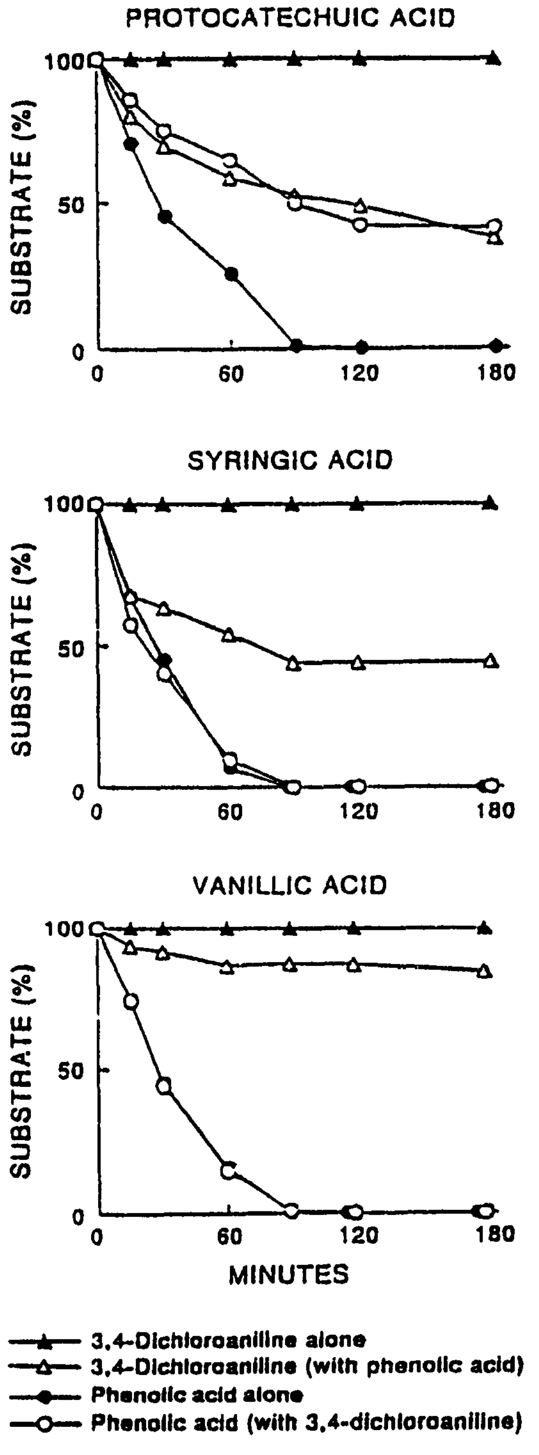


Figure 7. Transformation of 3,4-dichloroaniline and a phenolic acid (alone and in the presence of the other substrate) by a laccase of *Rhizoctonia praticola* (after Tatsumi et al. 1994).

Sorption

Katayama et al. (1995) remediated a soil in which the degradation of the fungicide chlorothalonil was carried out by adding farmyard manure at a dose of 400 tons (wet weight, ca. 25% dry matter) per ha. This amount is about a 10-fold of what is normally applied. In such case, the soil has to be plowed regularly in order to keep it aerobic. Yet, the overall costs involved were quite reasonable, i.e. 100.000 BEF (3300 USD) per ha. This corresponds with some 30 BEF ($\approx$ 1 USD) per ton soil treated.

Kästner et al. (1995) studied under laboratory conditions the fate of ^{14}C-labelled anthracene and hexadecane in compost-manured soil. They added mature compost at a dose of 20% dry weight per soil dry weight (i.e. equivalent to some 600 ton wet weight per ha). They noted that in a period of 100 days, some 24% of the ^{14}C-labelled anthracene was transformed to CO_2, about 42% became bound to the soil matrix and about 5% was recovered in the original state. In the soil without compost amendments, little changes occurred in that time period. The authors suggest that this approach offers potential and that further research should be done concerning the hypothese that a non-extractable pollutant can be considered as a non-pollutant.

In Europe, source separate selection of vegetable, garden and fruit wastes (VGF) has become an established practice and high quality VGF- compost is at present available in sufficient quantities (Gellens et al. 1995). The intensive use of this type of material in soil clean-up, particularly in the context of its capacity to bind pollutants, is justified. The current prices of high-quality compost are of the order of 1000 BEF (33 USD) per ton dry weight. A soil thus dosed at a rate of 200 kg dry weight compost per ton dry weight soil corresponds with a cost of some 200 BEF (6 USD) per ton of soil treated. There is substantial room of research and development to make compost more efficient (e.g. by increasing its binding capacity), more site compatible (e.g. by adjusting its levels of leachable minerals), and more environmentally sustainable (e.g. by augmenting its pollutant specific microbial populations).

The amount of organic matter added might make the soil too rich in nutrients and thus cause pollution of the groundwater with mineral nutrients such as nitrate and phosphate. Therefore, the possibility of adding a plain sorptive agent such as activated carbon can be examined. Indeed, activated carbon as used e.g. in the drinking water technology exhibits major sorption

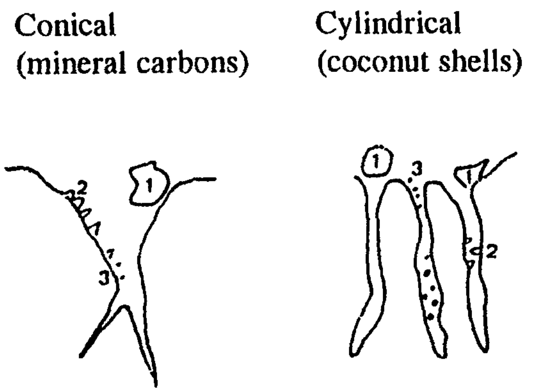


Figure 8. Pore structure (schematic). 1, Colloid or polymeric molecule; 2, Large adsorbed molecules (e.g., detergents, dyes, humic compounds); 3, Small adsorbed molecules (e.g., solvents, iodine) (Masschelein 1992).

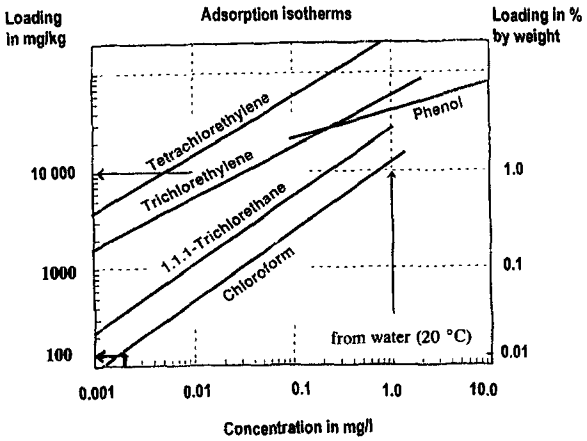


Figure 9. Adsorption isotherms for Hydraffin 30 (after Hydraffin®) powdered and granular activated carbon for water treatment.

capacities (see also Table 1). The most important parameter is the pore structure of the adsorbent (Figure 8). Two different types of structure are to be distinguished with regard to the pores: the cylindrical type (e.g., most activated carbon obtained from coconut shells) and the conical type (mineral carbons). At equal pore volume, the latter structure is more efficient for simultaneous adsorption of big and small molecules, while the former is often ineffective when obstruction occurs at the entrance with a large molecule or a colloid particle.

Activated charcoal has been widely investigated for detoxification purposes. Lamoureaux et al. (1989) investigated the amount of activated charcoal required to remove the herbicide norflurazon from a sand matrix. They found that addition ratios ranging from 100–300:1 activated charcoal:norflurazon were effective in completely reducing injury to corn, soybean and cotton. To remove organic herbicides from soil, amounts ranging from 50 to 400 kg of activated carbon for each 1 kg a.i. of herbicide were required. Ratios of activated charcoal:herbicide as high as 3600:1 have also been reported (Bovey & Miller 1969). At a first rule of thumb, a ratio of 10 000:1 appears quite safe; this corresponds with a loading for the activated carbon of 100 mg pesticide per kg sorbens. If one considers that common agricultural practices use some 3 kg pesticide active ingredient per ha (= 3 000 000 kg top soil), the latter approximation suggests that 30 000 kg activated carbon per ha or 10 kg activated carbon per ton soil can constitute an effective measure of remediation against pesticide residues.

It is of interest to refer to experiences with surface adsorption of organic pollutants in water treatment.

In the application of activated carbon adsorption to water treatment, multicomponent systems are usually encountered. In such a case the different compounds compete with each other for adsorption sites with the resulting overlapping and displacement effects. Consequently, the adsorptive capacity for any individual compound decreases in a solution when several compounds are present. The better the adsorption behaviour of the other compounds, the lower will be the selective adsorptive capacity for the individual compound. In this case, the gradient of the adsorption isotherms for each individual compound is determined by the concentration and adsorption behaviour of the other compounds present in the mixture. Here, the Freundlich equation is of limited use for determining the isotherm of the individual compound. Calculation on the effect of component interaction on the selective adsorption behaviour is complex and presupposes that the composition of the mixture and the adsorption isotherms of the individual components are known.

Figure 9 illustrates that many organic pollutants can be removed down to a concentration of 1 mg/l while sorbing more than 10 000 mg per kg weight onto the activated carbon matrix. Petroleum hydrocarbons and BTEX (benzene, toluene, ethylbenzene and xylenes) are for instance considered to no longer require active remediation when their levels in the groundwater decrease below 1 mg/l (Brown et al. 1995). If one requires the available concentration in solution to become below 1 μg/l, as in the case for pesticides, Figure 9 suggests that the permissible loading, i.e. the amount sorbed on activated carbon, will be a factor 100 times lower. Hence, at the permissible available water concentrations for pesticides ($< 1\ \mu$g/l), to assure safe conditions an amount of activated carbon must be supplied to the soil in that way that not more than 10 mg pollutant is loaded per kg powdered active carbon (PAC). This corresponds with the data discussed above (see e.g. Table 3).

For pollutants with permissible concentrations of ca. 1 mg/l in the water phase, one can thus propose that a sorptive loading of ca. 10 000 mg pollutant per kg PAC-sorbent is a reasonable assumption. For soils polluted with 1 g of hydrocarbon per kg, some 100 kg of PAC per ton of soil would be capable to bind the pollutant by sorption. This represents cost figures of the order of some 3000 BEF (≈ 100 USD) per ton of soil treated which is high but not unreasonable. However, the main question in this respect is how fast these sorption kinetics in soil will occur. Indeed, the goal is the slowly reversible sorption corresponding with the K_{app} defined by Pignatello and Huang (1991). Research should focus in this context on the methods to enhance the 'aging' processes in soils.

Considering the concept that 'aging' must be interpreted as diffusion into more remote surface sites, intensive mixing of soil and sorptive material will be essential. Obviously, mechanical mixing of surface and subsurface soils with materials such as powdered activated carbon can be achieved at reasonable costs. Yet, it is doubtful to what extend this will suffice considering the nanometre pores of activated carbon. In this context, the use of earthworms to mix the soil and the added sorbent might be of technological use in some particular cases. Indeed, earthworms intimately mix the inorganic particles with organic material in or on the soil, as well as nutrients (Devliegher & Verstraete 1995, 1996). Especially epigeic earthworms – small earthworms who live specially in or around organic matter at the soil surface – are good candidates. These epigeic earthworm species are very mobile and, according to Shipitalo et al. (1988), they can ingest and excrete up to 5 g soil DW g^{-1} earthworm DW d^{-1}. Moreover, during migration in soil, aeration channels are created, which are promoters for a good bioremediation of the soil. From our own experiments, we observed that *Dendrobaena veneta veneta* could resist a mineral oil content in the soil as high as 7 g kg^{-1} (Devliegher and Verstraete, unpublished data).

Chemical binding

As proposed by Shannon and Bartha (1988) and Bollag (1992) the decontamination of soil using enzymes which chemically bind the organic pollutant to the organic matter is obviously a promising strategy. However, only a limited group of such chemicals appear susceptible to such covalent linking processes. Field et al. (1995) give an overview of the molecules which are especially susceptible to polymerisation. Phenols in general, chlorophenols, catechols and tannins in particular, aromatic amines and nitroaromatics are susceptible to polymerisation under aerobic conditions. Sometimes, substantial amounts of copolymerizing agents such as phenolic compounds need to be added, together with the enzymes. The commercial availability and the cost price of the enzymes must be considered. Moreover, the outcome of the cross-linking detoxification can be variable (Aitken et al. 1994). All these aspects suggest that such an approach will be quite costly and moreover requires careful scrutiny with regard to the residual clean-up. Although the kinetics are promising, the practical barriers are considerable. On the other hand, Berry and Boyd (1985) proposed another potential approach. They demonstrated that the indigenous enzyme activity may be enhanced by the addition of ferulic acid and H_2O_2. They treated soil contaminated with 3,3′-dichlorobenzidine (DCB). So whereas addition of enzymes to soil may be economically prohibitive, addition of these inexpensive, naturally occurring reagents could be feasible from an economical point of view and thus, the indigenous oxidative-coupling activity could be enhanced. In contrast with surface adsorption, chemical covalent binding of organic pollutants offers no short term perspectives.

R&D needs

Bound residues in soil, or expressed in a more straightforward way, moieties of the original chemical compound remaining after exhaustive sequential extraction with non-polar organic and polar solvents, are regarded as constituting no immediate ecotoxicological danger. Consequently, the EU legislation has accepted the fact that for pesticide molecules, a major part can be transformed to non-extractable residues without representing direct harm to the environment. In view of the fact that many recalcitrant pollutants are present in heterogeneous mixtures and consequently are difficult to remove through high rate bioremediation technologies, it appears interesting to opt for a combined action. The first action consists of binding the major amount of available pollutant by means of slowly reversible surface adsorption mechanisms. For pesticides, the amount of organic matter present in the native soil may suffice to bind the residue. For more heavily present organic compounds, additional adsorption capacity should be provided. This can be achieved by mixing the soil with sorptive materials such as humified organic matter (compost, farm yard manure, . . .) at 100–200 kg per ton of soil or activated carbon at a dose of 50–100 kg per ton of soil. The second phase is a natural microbial degradation of the pollutant which is slowly set free in the aqueous phase.

Several areas for research can hereby be delineated. First, there is the need to engineer effectively and rapidly binding of pollutants to sorptive phases such as compost and activated carbon (powdered or granulated) in a soil environment. A major aspect hereby is the engineering of contact between the pollutant and the sorbent. Both mechanical and biological routes have to be developed to obtain intimate mixing of soil and sorbent. A second aspect is the quality criteria to be imposed on the compost used. As indicated before, dissolved humic substances present in compost can enhance the desorption of hydrophobic pollutants attached to soil (Janzen et al. 1996; Rebhun et al. 1996). Hence, the compost should not only be of high quality in terms of its content in heavy metals and other possible contaminants, but it should also be defined in terms of soluble humic substances, relative to the technology of application and soil remediation. The pathways and kinetics of so-called 'intrinsic' or 'natural' biodegradation of bound residues need to be better documented (Brown et al. 1995). Thirdly, the microbial degradation processes of bound residues need to be quantified. The studies by Volkering (1996) emphasize that the main limiting factor is the mass transfer from the matrix to the solution. There are indications that degradation of non-dissolved molecules can occur and this aspect deserves much more focus. Moreover, the compost should not be too rich in leachable minerals, particularly nitrate. Compost resulting from anaerobic processing followed by aerobic maturation is reported to be low in mineral N and can thus offer an opportunity (Gellens et al. 1995). It should be noted that certain countries have stipulated maximum application rates of the order of 6 ton of compost per ha per year (De Haan & Van der Zee 1996). In such cases, appropriate combinations of compost, PAC and other sorbents might be required by the regulator. Finally, the ecotoxicology of the bound residues requires better understanding, particularly under worst-case conditions such as leaching or ingestion. The latter obviously are of major concern but they should not prevent regulators and contractors to opt for approaches which offer possibilities for remediative action (Verstraete et al. 1995).

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