

MICROBIAL CONTROL OF PESTICIDE PERSISTENCE IN SOIL

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Summary The multiplicity of microbial factors which determine pesticide longevity in soil is reviewed. These include metabolic and co-metabolic activity, exoenzymic catalysis, immobilization, and incorporation into humus. A thorough knowledge of these influences may form the basis for practices which attenuate pesticide persistence in soil. For example, manipulating the soil pH and oxygen level will often influence degradation by quantitatively and qualitatively changing the microbial population. Addition of organic matter may induce a similar response. The availability of pesticides to indigenous microorganisms can be altered by the addition of adsorbents, whilst irrigation and ploughing will also modify breakdown rates. The interesting possibility of microbial or enzyme soil additives is discussed.

INTRODUCTION

The World's expanding population has ensured an increasing dependence on agrochemicals to enhance food production. Admittedly genetic manipulation, wider use of fertilizers and improvement in land management have all contributed to increases in yield, yet pesticides and modern agriculture have become inseparable.

For the safe and most effective use of any pesticide it is essential to know how long it will remain active against the target organism and whether its breakdown products will have any deleterious effect on other components of the biosphere. In an agricultural Utopia all pesticides would be specific for the job in question and then rapidly degraded to non-toxic metabolites. Unfortunately the persistence and toxicity of many pesticides is difficult to forecast and considerable environmental disruption has arisen from their indiscriminate use.

The subject of pesticide interaction with, and loss from soil has been widely discussed in recent years (Edwards, 1974, Green, 1974, Weber and Weed, 1974, Burns, 1975) as have attempts to quantify their behaviour (Hamaker, 1972, Adams, 1973, Walker, 1974). Most pesticides disappear from the soil environment through a combination of routes including volatilization, microbial and non-microbial decay, leaching, surface run-off, plant uptake and, more subtly, involvement in food chains. The choice of route varies with the chemical and physical properties of the pesticide, its mode of application, climatic conditions and an host of soil biotic and abiotic factors (Table 1). This paper reviews the ways in which microorganisms influence pesticide behaviour and suggests how this knowledge may be applied to the control of persistence in soil.

Table 1

Soil Factors Affecting Pesticide Loss

Abiotic	Biotic
sand	bacteria
silt	fungi
clay	protozoa
metallic oxides	invertebrates
pH	plant roots
water	organic matter
temperature	exoenzymes
oxygen	

THE INFLUENCE OF MICROORGANISMS ON PESTICIDE PERSISTENCE

Microorganisms affect pesticide persistence in a variety of direct and indirect ways such as substrate utilization, co-metabolism, exoenzyme production, immobilization, and incorporation into humic material.

1. Microbial Metabolism.

Microbial degradation is critical to the fate of many pesticides in soil. Notwithstanding, microbes are far from infallible in their ability to degrade each and every substrate presented to them and some pesticides appear completely resistant to microbial attack. These recalcitrant chemicals include those, such as parquat, that the soil environment renders unavailable to microbes (Knight and Tomlinson, 1967) and those that are not susceptible to microbial degradation under any conditions (Kaufman and Plimmer, 1972). It is, of course, sometimes difficult to irrevocably establish that microbial degradation is the sole or even a partial influence on pesticide loss. Traditionally persistence in autoclaved soils and disappearance in non-sterile soils was adequate to support the idea of microbial decay. However, autoclaving is known to radically change the physical and chemical characteristics of the soil in addition to removing the microorganisms. These changes may affect other mechanisms of pesticide decay. Nowadays less drastic methods of eliminating microbial activity are also used and include gamma irradiation and the addition of microbial inhibitors (toluene, sodium azide). Notwithstanding, enrichment and isolation of particular microbial species may be the most convincing way of demonstrating (and studying the biochemistry of) the biological breakdown of pesticides.

Microorganisms, of course, recognise pesticides as carbon-, nitrogen- and energy-containing substrates and the *in vitro* decay of many of them is well described (Kaufman 1974, Wright 1974, Higgins and Burns 1975). Additionally, as in the break-

down of naturally-occurring organic matter, substrate specificity exists in pesticide-microbe interactions. In other words a particular species or group of species are capable of degrading one pesticide whilst leaving another untouched. In the broadest sense some pesticides are broken down most effectively by fungi or bacteria or actinomycetes whilst others are degraded by aerobes or anaerobes. As a result, environmental conditions (such as pH and water content) which favour certain groups of microorganisms will, in turn, effect the breakdown of certain pesticides.

2. Co-metabolism.

In addition to direct metabolism of pesticides microbes can also induce breakdown without deriving any carbon or energy from the reaction. This process is called co-metabolism or co-oxidation and has been reviewed recently by Horvath (1972) and Alexander (1973). For co-metabolism to occur the microbes must obtain the bulk or all of their carbon and energy from other substrates. The phenomenon of co-metabolism is extremely important because it presents the possibility that a series of reactions involving several different microbial species, some behaving metabolically, others cometabolically, can lead to the total decay of a pesticide. For example, there is evidence to suggest that DDT (Focht and Alexander, 1970), 2,4,5-T and 2,3,6-TBA degradation is a combination of metabolism and co-metabolism.

It is possible that co-metabolism is a common and important mechanism of pesticide decay but that its operation is disguised by the microbial heterogeneity of the *in vivo* soil environment.

3. Exoenzymes.

Soils have an indigenous enzymic activity composed of both intra- and extracellular fractions. A proportion of the extracellular fraction is extremely persistent due to its association with the soil clay and organic matter colloids. Recently it has been shown that some exoenzymes are capable of degrading pesticides without relying upon proliferating microorganisms. Indeed in soil and soil extracts free of microorganisms biological decay of pesticides will occur. Satyanarayana and Getzin (1973) isolated a stable esterase fraction from soil which hydrolysed the organophosphorus insecticide, malathion. This catalyst resisted gamma irradiation though not autoclaving, was extremely persistent and showed typical enzyme kinetic characteristics.

We have further examined malathion esterase and the breakdown of malathion in soil (Tables 2 & 3) and have found the enzyme to have similar properties to those of urease. We have previously suggested (Burns, Pukite and McLaren, 1972) that urease functions extracellularly from within the organic matter of the organo-mineral complex. This location allows the diffusion of substrate and product molecules to and from the enzyme, but immobilizes and protects the enzyme itself.

Although little other research has been carried out on pesticide-degrading stable exoenzymes the malathion esterase story is of interest because it describes an hitherto unknown method for the degradation of pesticides in soil.

4. Immobilization.

Occasionally the fate of a pesticide may be inadvertently influenced by microorganisms which absorb the molecule but are unable to degrade it. The pesticide may then remain essentially unchanged in its intracellular location until the death and lysis of the bacterium. More seriously, perhaps, the pesticide-microbe combination may become involved in food chains. It has been suggested that immobilization in this manner is one of the contributory factors in DDT persistence and biomagnification.

Table 2

Breakdown of Malathion in soil

<u>% remaining at day:</u>	<u>non-sterile</u>	<u>irradiated (2.5Mrads)</u>	<u>autoclaved</u>
1	43	82	96
2	25	40	92
3	17	27	100
4	12	9	96
7	8	10	100
9	8	10	93
11	5	6	98
15	4	1	100
17	3	3	95

Table 3

Breakdown of Malathion in soil components

<u>% remaining at day:</u>	<u>clay</u>	<u>sand</u>	<u>silt</u>	<u>organic matter</u>	<u>organo-mineral complex</u>
1	97	95	98	88	42
2	97	94	99	43	25
3	91	93	91	18	15
4	87	94	81	5	9
7	91	73	54	0	5
9	81	61	55	0	4
11	89	50	28	0	2
15	69	38	19	0	2
17	56	24	14	0	2

5. Incorporation Into Humus.

Soil humic matter which, after all, is itself a product of microbial decay and synthesis, is frequently described as "the most important single factor affecting pesticide persistence". Soil organic matter may immobilize pesticides by acting either as an highly anionic adsorptive surface or as a lipophilic moiety attracting many non-ionic pesticides. Pesticides may also become chemically and physically trapped within colloidal organic matter during its formation by microorganisms (c.f. enzymes, above), an association which may protect them from the normal mechanisms of decay. The longevity of methoxychlor may be influenced in this way (Mathur and Morley, 1975), as may the chloraniline residues of acylanilides, phenylcarbamates and phenylurea herbicides (Hsu and Bartha, 1974) which after incorporation into soil

humus are only slowly available to microbial decay. In contrast the biochemical breakdown of pesticides, such as atrazine (Li and Felbeck, 1972), may be catalysed by humic matter. A constant level of organic matter is, of course, essential to support an indigenous microbial population. This is one of the reasons why microbial numbers are low and subsequent decay of pesticide is often slower in sandy soils (< 1% organic matter) than in soils with higher levels of colloidal organic matter. The immense variety of roles played by organic matter in pesticide behaviour has been summarised by Burns (1972).

THE CONTROL OF MICROBIAL ACTIVITY

It is possible to influence the persistence and toxicity of a pesticide by adjusting its relationship to the microbial population of the soil. It is fair to say that in some instances the practical value of the methods involved is far from established and, in others can only be a source of speculation. Nevertheless, the potential benefits are enormous and research is likely to increase significantly in the next decade.

1. Changing to Physical Environment of the Soil.

The knowledge that certain microorganisms are specific for particular pesticide substrates can be used to modify the soil environment in an attempt to stimulate desirable segments of the microbial community. The two adjustments most frequently examined are to pH and oxygen levels.

Minor pH changes (with lime or sulphur additions) may radically alter the balance of the microbial community. In very broad terms a shift to alkalinity will encourage the three major soil actinomycete genera *Streptomyces*, *Nocardia* and *Micromonospora*; a drop in pH will support the acidophilic fungi; whilst bacteria tend to favour neutral soils.

As observed previously, some recalcitrant pesticides are degraded quite rapidly by anaerobic organisms. For example DDT, lindane, heptachlor, endrin (Guenzi, Beard and Viets, 1971) and methoxychlor (Castro and Yoshida, 1971) are broken down much more readily under anaerobic conditions than in aerobic conditions. In the soil pores water competes with oxygen for space, the concentration of one is inversely proportional to the other and thus the fastest way of inducing anaerobiosis is to flood the soil. As this may be acceptable practice in some areas (rice fields, alkaline soils) the short term use of organo-chlorine insecticides may be compatible with existing agricultural practices. At the other extreme water will encourage microbial activity in arid soils.

A second method of creating anaerobic conditions in soil is to add an excess of easily degradable organic matter. This will stimulate microbial activity which will, in turn, consume oxygen faster than it can diffuse from the atmosphere into the soil solution. Under these conditions anaerobes and facultative anaerobes will soon dominate the community.

2. Addition of Organic Matter.

Microbial numbers can be increased by organic matter amendments. The result of this treatment may be a more rapid decay of pesticides, either because an active, high density microbial population is more likely to contain species capable of decaying 'exotic' substrates or that the enriched population will turn its attention to alternative sources of carbon, nitrogen, and energy once the added organic matter is used up. This stimulation of breakdown of one type of organic substrate in the

presence of another is well known in natural organic matter decay. For example, the presence of hemicellulose speeds up the decay of the more biologically resistant cellulose. The carbon to nitrogen ratio must also be considered and the addition of ammonium nitrate will usually satisfy this requirement. McLure (1970) investigated the effect of applying nutrient and Czapek Dox broths to herbicide-amended soil. Plant bioassays indicated that phytotoxicity (and probably degradation) was significantly affected (Table 4). These results suggest that field applications of broths, which will stimulate all or just specific groups of microbes warrant further investigation.

Table 4

Effect of microbial broths on herbicide degradation (After McLure, 1970)

Herbicide	% change in foliar yield	
	Czapek Dox	Difco Nutrient
Diphenamid	+250	+90
Monuron	+50	-67
Atrazine	+40	+90
Dicamba	+53	+15
CIPC	+51	+13
Amiben	+91	-25
No herbicide control	+27	+6

Organic matter may also encourage the rate of plant root uptake (Minshall, 1969) and if used in conjunction with those plants which can absorb high quantities of pesticides may serve as one method of decontaminating soil. Additionally the increase in nutrient levels may aid the plant to outgrow any pesticide effect.

Foliar feeding with organic matter sprays, such as urea, will often stimulate rhizosphere populations, a phenomenon which may prove a profitable research area because it is in the root region of the plant where detoxication is most critical.

3. Enzyme Additions.

The importance and possible applications of bound exoenzymes in pesticide breakdown in soil can, as yet, only be guessed at. But if the experience of malathion is at all common then the addition of specific catalysts to soil is a promising avenue of investigation. The enzymes would need to be complexed with a natural or artificial colloid complex (Ladd and Butler, 1975) to impart resistance to the denaturation pressures of the soil environment. We have found that as little as 5% b/w of an enzyme-organo-mineral complex will significantly stimulate the decay of malathion.

4. Altering Pesticide Availability.

The breakdown and effectiveness of a pesticide in soil is closely akin to its adsorptive properties. The processes involved in adsorption have been extensively reviewed elsewhere (Bailey and White, 1970) but it is as well to remember that although adsorption may delay breakdown in many instances it may also stimulate

decay in others (Burns, 1975).

Adsorption and desorption (and thus availability to microbes and enzymes) is significantly influenced by pH. Comparatively minor pH changes can alter both the nature of the adsorbant and the adsorbate. In general terms, the more acid the environment, the greater the dissociation of ionic pesticides and the higher the likelihood of adsorption through such mechanisms as hydrogen bonding or protonation. More directly, breakdown of a pesticide can sometimes be decreased by the actual addition of adsorbents such as clays and charcoal (Moyer, Hance and McKone 1972), resins and organic matter, either to the soil in general or by planting seeds in a pocket of adsorbent (Kratky and Warren, 1971).

Alternatively microbial inhibitors such as toluene, sodium azide and a variety of antibiotics, could be employed to retard decay. However, the broader effects of large scale microbial inhibition on biogeochemical cycles would have to be considered.

The use of irrigation water to transport pesticide from soil horizons of high to those of low microbial activity (Table 5) may also be important. Atrazine is degraded 2-3x faster in top soil as in sub-soil (Roeth, Lavy and Burnside, 1969) although this breakdown is not the entire responsibility of microbial activity.

Table 5

Distribution of Microorganisms in a soil profile (Starc, 1942)

Horizon	Depth(cm)	Organisms. g soil ⁻¹ (x 10 ⁻³)				
		Aerobic Bacteria	Anaerobic Bacteria	Actinomycetes	Fungi	Algae
A ₁	3- 8	7800	1950	2080	119	25
A ₂	20-25	1800	379	245	50	5
A ₂ -B ₁	35-40	472	98	49	14	0.5
B ₁	65-75	10	1	5	6	0.1
B ₂	135-145	1	0.4	0	3	0

5. Cultural Practices.

Fallowing land tends to conserve moisture, tillage operations increase aeration, both characteristics conducive to pesticide breakdown by microorganisms. Regular cultivation may decrease pesticide residues in soil (Lichtenstein and Schulz, 1961) although deep ploughing may remove the pesticide from microbially active surface soils and inhibit subsequent breakdown.

6. Prior Pesticide Treatment.

It has been demonstrated many times, since the heuristic work of Audus (1951) and others a quarter of a century ago, that treatment of a soil with a pesticide will stimulate the breakdown of successive applications of that pesticide. For example, successive supplements of phenoxyacetic acids, such as 2,4-D and MCPA will reduce the lag phase of microbial breakdown until degradation begins almost immediately following application. It is further known that this is a long-term effect, the induction surviving some 6-12 months after the initial application of herbicide. Microbial enrichment of this type is not necessarily limited to one pesticide and

cross-adaptation may also occur. For instance, Tortensson, Stark and Laranson (1975) have indicated that MCPA will enrich a microflora that will stimulate the decay of 2,4-D (and vice versa) whilst McLure (1974) has shown that a range of soil microorganisms (*Fusarium*, *Penicillium*, *Arthrobacter*) will degrade a wide range of anilides after adaptation to prophan as their sole carbon source. Fryer and Kirkland (1970) reported that MCPA persistence was inversely related to the number of previous applications.

7. Microbial Additions.

To many of those involved in pesticide research, the addition of suitable microorganisms to soil to stimulate breakdown is the most exciting control method. However, before endorsing the practice one has to consider the underlying ecological principles (Brown, Jackson and Burlingham, 1968). The selection pressures in soil are so extreme that, in the climax microbial community, almost every available ecological niche is filled. Thus additions of microorganisms to soil may be doomed from the start and over the years attempts to increase such parameters as nitrification and phosphate availability have met with only limited success and much controversy. Notwithstanding, if a new microenvironment is created it may be possible to pre-empt natural colonization with the use of a microbial inoculum. Successful examples of this include the symbiotic association between legume and rhizobia and the mycorrhizal structures essential for the growth of conifer seedlings. Therefore, with an overall understanding of microbial ecology, the stimulated breakdown of a novel substrate, such as a pesticide, is certainly worth investigating.

Kearney *et al* (1969) were able to accelerate the degradation of DDT in flooded soils by the addition of *Aerobacter aerogenes* and some breakdown of the highly cationic herbicide pesticide has been reported in soils inoculated with the yeast *Lipomyces starkeyi* (Burns and Audus, 1970). It has recently been suggested that an *Arthrobacter* soil inoculum may accelerate the decay of a linuron-phenylmercury acetate pesticidal mixture (Balika, Kasinkiewicz and Stankiewicz, 1974). Of course, large scale inoculation, were it ever deemed feasible, presents considerable economic (and possibly health) problems. A less drastic approach may involve coating plant seeds with suitable degradative microorganisms (Maxfield, 1969) or even the introduction of small quantities of selected microbes which may then transfer their degradative ability to the indigenous population (Waid, 1972).

CONCLUSION

This review has described some of the possible methods of controlling pesticide persistence through biological activities. Many of these methods are curative rather than preventative and it is perhaps this drawback which should guide future research. It would appear more satisfactory to choose a pesticide for a specific task than to attempt to alleviate any subsequent residual effect. In the long term only a thorough knowledge of the chemical, physical and biological factors affecting persistence will allow this predictive approach to pesticide use. More immediately, the retardation of breakdown of non-persistent pesticides may prove the most rewarding (and certainly safest) approach and the use of microbial inhibitors, adsorptive agents and even pesticide-colloid complexes is promising. In situations where recalcitrant pesticides are a problem, less susceptible crops and alternative cultural techniques should be considered (see Lebaron, 1970). The short term changes of the soil environment by flooding, liming or organic matter incorporation also warrant attention, whilst the addition of microbial inocula has the most potential but may eventually prove the most disappointing.

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