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3.2 Biotic Degradation of Pollutants

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Pollutants in the environment are exposed to various degradative forces. Among them biotic degradation, or metabolic processes, are known to play a vital role in deciding overall fates of organic pollutants. They not only contribute to the disappearance of the original form of pollutants, but also change their physico-chemical properties, and thus affect their transport and distribution behaviour among various compartments in the environments.

Most forms of living organisms are capable of directly interacting with pollutants, and some of them are capable of metabolizing even very recalcitrant pollutants. Metabolic processes found in microorganisms, plants, and animals are, however, qualitatively different.

Microorganisms are known to play major roles in metabolizing chemicals in the environment (Hill and Wright 1978; Matsumura and Krishna Murti, 1982). Their contributions to the metabolic alteration of pollutants in the environment are aided by the phenomenon that the bulk of pollutants are found in soil and aquatic sediment (excluding open ocean floors) loaded with microorganisms. Therefore, in this section, a great emphasis will be given to microbial degradation of pollutants. This does not imply that other types of living organisms do not contribute to overall metabolic activities. They indeed do play major roles. However, the emphasis of this section is on the metabolic events occurring on pollutants before they enter food chains and the effects of climate on such processes. Since most plant and animal species may be considered members of food chains, their exclusion from this section may be justified. The reader who is interested in animal and plant metabolism of pollutants is referred to more specialized textbooks (e.g., Matsumura and Krishna Murti 1982).

3.2.1 CHARACTERISTICS OF MICROBIAL METABOLISM

It was originally assumed by many pesticide scientists that the patterns of microbial metabolism were in general very similar to the ones already found in animals, particularly the mammalian species, as studies on microbial metabolism of pesticides were lagging far behind comparable studies in mammalian species. However, as knowledge on microbial degradation has advanced, it has become apparent that in many cases the patterns of degradation in these two different groups of organisms are often very different.

First of all, the purpose of all metabolic reactions on xenobiotics in higher animals is to eventually convert them into polar and, therefore, excretable forms. Second, in higher animals the processes of primary metabolism of xenobiotics are centralized in a few specialized organs. In the case of the liver, its metabolic pattern is largely determined by the activity of an oxidative detoxification system, generally termed mixed-function oxidase.

On the contrary, the predominant metabolic activities in the microbial world are meant for production of energy. In this respect, it is not even possible to define xenobiotics here, since most organic materials can serve as the source of energy to at least some microorganisms. Hence only a few groups of chemicals may be regarded as foreign to microorganisms. Among insecticidal compounds, halogen-containing chemicals, particularly halogenated aromatics, must be regarded as generally foreign (or unusable) material to microorganisms.

Another characteristic of microbial metabolism is the adaptability of microorganisms to changing environments through mutation and induction, particularly toward chemicals that are initially toxic to them. The case of penicillin resistance in bacteria through induction of penicillinase is well known.

The metabolic activities of microorganisms encompass many different types of biological processes not found in any other organism. They include fermentation, some anaerobic metabolism, chemolithotrophic metabolism, and metabolism through exoenzymes.

In general, microbial contributions to metabolic alteration of insecticides may be classified in several categories, as shown in Table 3.2.1. It must be stressed that the main purpose of such a classification effort is to present clearly the types of microbial degradation according to their final manifestations. They are not classified according to the intrinsic mechanisms by which they degrade pesticides. Various reactions which involve different enzymatic mechanisms and yet are known to behave in similar patterns have been grouped together.

3.2.2 WIDE-SPECTRUM METABOLISM: INCIDENTAL METABOLISM

The key characteristic of incidental metabolism is that the pesticides themselves, including any part of the pesticide molecules, do not serve as the energy and carbon source for the microorganisms. Therefore, addition of pesticides does not affect their growth, which is always controlled by other nutrients. Two completely different subgroups of metabolic activities are present in this type. In the first subgroup, pesticides are degraded by enzymes which are not specifically related to pesticidal molecules (Table 3.2.1, categories a and b).

Table 3.2.1 General classification of microbial metabolism of pesticides. Modified from Matsumura and Krishna Murti (1983)

ENZYMATIC

(A) Incidental metabolism: pesticides themselves cannot serve as energy sources
- (i) Wide-spectrum metabolism: metabolism by generally available enzymes
  - (a) Metabolism due to generally present broad-spectrum enzymes (hydrolases, oxidases, etc.)
  - (b) Metabolism due to specific enzymes present in many microbe species
- (ii) Analogue metabolism (co-metabolism)
  - (c) Metabolism by enzymes utilizing substrates structurally similar to pesticides

(B) Catabolism: pesticides serve as energy sources
  - (d) Pesticides or a part of the molecule are the readily available source of energy for microbes
  - (e) Pesticides are not readily utilized. Some specific enzymes must be induced.

(C) Detoxification metabolism
  - (f) Metabolism by resistant microbes

NON-ENZYMATIC

(A) Participation in photochemical reactions
(B) Contribution through pH and other physico-chemical changes of microenvironment
(C) Through production of organic and inorganic reactants
(D) Through production of cofactors

In these cases pesticides are degraded by either broad-spectrum enzymes, such as hydrolases, reductases, and oxidases, or by specific enzymes commonly present in high percentages of microorganisms. In either case the pesticidal substrates are metabolized as a result of general microbial activities. In the environment, therefore, those types of reactions are observed in which microbial activities are stimulated by the availability of nutrients and moisture, and by the right temperature and pH.

3.2.3 ANALOGUE METABOLISM: INCIDENTAL METABOLISM

The term co-metabolism has been used in the past to include all types of metabolism in categories a, b, and c (Table 3.2.1). However, in this section I have decided to apply the term in a more restricted manner to include only the cases where the microorganisms are induced by chemicals which structurally resemble the pesticide molecules. Thus, to avoid confusion, I shall use the term analogue-induced metabolism here. In such a case the microorganisms can grow on the given chemical (analogue) but not on the pesticide, despite their capability to at least partially metabolize the latter. Good examples are those produced by Focht and Alexander (1970a, 1970b), who obtained DDT-degrading *Hydrogenomonas* sp. (*Pseudomonas* sp.) by using diphenylmethane as a carbon source, and those produced by Ahmed and Focht (1972) and Furukawa and Matsumura (1975), who selected PCB-metabolizing microorganisms by using biphenyl as a carbon source. The important feature distinguishing this type from other incidental metabolisms is that these microorganisms are purposely selected by non-pesticidal analogues. The enzyme systems induced do not initially recognize the difference in the substrate molecules and, therefore, partially degrade the pesticide molecules. However, in such cases the microorganisms are incapable of completing the metabolism necessary to receive energy for growth.

3.2.4 CATABOLISM

In catabolism microorganisms are capable of deriving energy from the pesticide molecules and, therefore, can grow on them. I include here cases in which only a part of the pesticide molecule is utilized for growth from a practical standpoint. Thus a complete mineralization of the pesticide is not the absolute requirement so long as growth is observed by using the pesticide as a sole carbon source.

3.2.5 NON-ENZYMATIC PROCESSES

The processes by which microbial activities contribute to the overall alteration of insecticidal molecules by non-enzymatic mechanisms are less well studied than those involving enzymatic reactions. It is known that some pesticidal chemicals can be photochemically altered in the environment, and microbial products can promote photochemical reactions in two ways. First, microbial products may act as photosensitizers by absorbing the energy from light and transmitting it to the insecticidal molecule. We have been able to show, for instance, that an aqueous extract from heat-sterilized blue-green algal cultures promoted photochemical degradation of DDT (Esaac and Matsumura, 1980). Another way that microbial products can facilitate such photochemical reactions is to serve as donors or acceptors of electrons and/or reacting groups of chemicals, for example, hydrogen and OH-, which are often needed for photochemical reactions.

Recently, Esaac and Matsumura (1980) demonstrated that ferridoxin and flavoproteins isolated from algae are powerful photosensitizers. These are known to play important roles in electron transfer systems in algae. Since they are quite stable molecules, it would not be surprising if they persist long enough in the environment after the death and lysing of algae cells to become a factor in pesticide degradation.

The effect of pH is often neglected in the field of pesticide metabolism despite numerous reports on the pH-dependent reactions of relatively labile molecules, both in soil and *in vitro*. Large pH changes are often associated with microbial activities, together with changes in nutritional sources, particularly in aqueous media. Initially degradation of proteins causes alkaline pHs, and with carbohydrate metabolism the pH becomes acidic. While the actual occurrence of microbial pH effects in nature might be difficult to document, it is certainly easy to demonstrate the phenomenon *in vitro*, where during the decay period the pH of spent culture media often becomes very low. Incubation of labile insecticides, such as tetraethyl pyrophospate (TEPP), with such spent medium under sterile conditions would certainly cause breakdown of the insecticides.

Little attention has been paid so far to the importance of the microbial formation of organic products capable of reacting with pesticides. Such reactants of microbial origin can be postulated to include amino acids, peptides, alkylating agents such as acetyl CoA, methylcobalamine, adeosylmethionine, and organic acids.

3.2.6 GENERAL EFFECTS OF ENVIRONMENTAL CONDITIONS ON SOIL MICROBIAL ACTIVITIES

Many environmental factors are known to affect the rate of metabolic conversion of pollutants. In the case of soil microbial activities, water content of soil greatly affects and in extreme cases limits the type of microbial activities. Waterlogged conditions in combination with high nutrients could promote growth of anaerobic microbial species. Extremely dry conditions limit microbial activities. The tendency is greatest with sandy soil, with least water-holding capacity among various soil types. In very dry conditions *Aspergillus* and *Penicillium* species show abilities to persist, while streptomycetes show moderate degrees of tolerance to dryness. Bacteria in general require high moisture, though some (e.g. *Bacillus* spp.) are capable of surviving dry periods in the form of spores.

Acidity also affects soil fauna. In general acidity depresses the growth of bacteria more than that of fungi. For instance, below pH 5 bacterial activities are usually very low.

Soil is generally considered to be nutrient deficient as a normal state (with some exceptions, such as forest soil). Therefore, addition of exogenous nutrients causes a sudden burst of microbial activities. Yet, increase in total microbial activities alone may not result immediately in increased metabolism of pollutants as will be explained later.

Temperature is the major contributing factor in deciding the characteristics of soil biota. Each microbial species has a definite range of temperature preference for significant activity. Thus composition of active microbial species varies in a given soil as temperature changes. For instance, Okafor (1966) has found that chitin metabolizing organisms in tropical soil at 28–30°C were predominantly actinomycetes, nematodes, and protozoans, while at 2–15°C, fungi and bacteria were more important. It must be emphasized, however, that higher temperature does not automatically guarantee higher microbial populations and activities. Certain microorganisms are well adapted to colder climates and as such would flourish even at seemingly low temperatures. In temperate zones with moderate to severe seasonal variations, for instance, it is common to observe maximum microbial activities in spring and fall rather than during the summer, even where adequate moisture is maintained throughout the year.

Other determinants, such as redox potential, nutrients, and physico-chemical characteristics of soil, also play very important roles. However, they play similar roles both in the tropics and temperate zones. Among these factors there are certain known joint actions. For example, the availability of nutrients with water often creates low redox potential, and at the same time, in those environments where nutrients and water are constantly available, the soil characteristics become organic.

In arid environments microbial activities are naturally low, even with irrigation, since soil lacks organic constituents. Under such conditions organic pollutants tend to vertically migrate to lower layers because of low holding capacity of sandy soil, where microbial activities are low, causing groundwater contamination where there is little microbial action.

3.2.7 COMPARISON OF PESTICIDE DEGRADATION PATTERNS BETWEEN TEMPERATE AND TROPICAL REGIONS

Despite the wealth of information on metabolic degradation of organic chemicals, mostly in temperate zones and to a lesser extent in tropical and subtropical regions, there is a striking absence of actual scientific data to indicate comparative differences in patterns of microbial degradation of organic chemicals. There are a number of inferences, suggestions, and generalizations regarding higher levels of microbial activities in tropical environments as compared to temperate areas and hence faster disappearance of organic chemicals in the former environments. Yet, there is little evidence to prove such a point.

In Table 3.2.2 I have summarized some of the pesticide residue data gathered for registration purposes in the USA under the IR-4 Program (Interregional Project No. 4). These are the cases where a given pesticide is applied to an identical crop under an identical application protocol in two or three regions with different climatic conditions within the USA. The resulting residues in the crop should, therefore, indicate the relative levels of pesticides remaining in the soil.

In the case of iprodione application on broccoli there was a striking difference between north and south in its residue levels. In Michigan, which is a northern state, the residue levels in broccoli were much higher than those found in Georgia despite the fact that iprodione was applied for fewer days. Yet, the tendency is not consistent. In the case of permethrin application on cantaloupe, the highest residue level was found in those samples from Texas at 0.4 pounds per acre (450 gm/ha). Under identical test conditions cantaloupe from Indiana, which is a northern state, showed a lower residue level. The fact that the same tendency is observed at 0.2 pounds per acre shows that this tendency is not a coincidence.

Table 3.2.2 Comparative residue data from some pesticide-crop combinations among various regions of the United States

Project Number	Pesticide	Commodity	State	PHI day	RATE lb/A	RESIDUE ppm	AVE. ppm
1545	Chlorpyrifos	Cantaloupe	IN	31	2.0	ND	<0.01
			TX	35	4.0	ND	<0.01
			CA	34	2.0	ND.-0.11	0.08
1545	Chlorpyrifos	Summer squash	IN	31	2.0	ND	<0.05
			CA	31	2.0	ND	<0.05
2194	Iprodione	Broccoli	MI	11	1.0	1.77–6.15	4.30
				11	2.0	3.41–11.94	6.81
			GA	17	1.0	0.03–0.06	0.04
				17	2.0	0.05–0.08	0.06
			CA	30	1.0	0.03–0.06	0.04
				30	2.0	0.03–0.05	0.04
1730	Permethrin	Cantaloupe	IN	1	0.2	0.26–0.57	0.39
				3	0.2	0.22–0.36	0.31
				5	0.2	0.17–0.49	0.29
				1	0.4	0.20–0.77	0.50
				3	0.4	0.57–1.42	1.00
				5	0.4	0.28–0.66	0.47
			TX	1	0.2	0.70–0.95	0.80
				3	0.2	0.30–1.31	0.91
				5	0.2	0.48–0.73	0.63
				1	0.4	1.51–1.92	1.73
				3	0.4	0.77–2.55	1.48
				5	0.4	1.26–2.67	1.75
			CA	1	0.2	0.13–0.41	0.22
				4	0.2	ND–0.35	0.17
				6	0.2	0.10–0.14	0.11
			FL	1	0.2	0.06–0.26	0.13
				3	0.2	0.11–0.24	0.16
				5	0.2	0.60–0.15	0.12
				1	0.4	0.39–0.61	0.48
				3	0.4	0.23–0.32	0.27
				5	0.4	0.17–0.41	0.27
1886	Monitor	Endive	NY	28	1.0	0.08–0.60	0.24
			FL	28	1.0	0.56–1.30	0.99

IR-4 pre-registration data (1984) for EPA. Compiled by Dr. R.A. Leavitt, Pesticide Research Center, Michigan State University, East Lansing, Michigan.

Since in these studies protocols in the field experiments as well as for the analytical procedures are rigidly defined and the quality control is achieved via sample exchanges, a direct comparison of the residue levels, and hence the probable rates of pesticide disappearance is possible. Upon examination of the entire data presented in Table 3.2.2, one is forced to conclude that there is no consistent trend to indicate that the disappearance of pesticides in one region is faster than that in other regions.

The foregoing discussion illustrates the difficulties and the problems associated with generalizing certain trends in microbial degradation of pollutants. The rates by which organic substances are degraded in soil and aquatic environments are governed by many complex environmental factors. Temperature, exclusive of extremes, is only one of these rate-determining factors.

Having failed to demonstrate simple trends among microbial degradation patterns in different climates, a question may be raised whether there could be other ways to approach the subject area. In the absence of truly comparable data among various climatic zones, one is forced to examine the data addressing seasonal variations in disappearance of organic substances (e.g. pesticides) in the same locality or experimental plot. This approach has merit in that the basic soil characteristics during the experimental period may be assumed to be relatively constant. The shortcomings of this approach are: (a) often temperature changes come with other climatic changes, such as humidity (e.g. rainy season), day length, and nutritional inputs; and (b) the findings from such studies may not be applicable where seasonal variation in climatic conditions is minimal.

Telekar *et al.* (1977; 1983a; 1983b) have studied persistence of several insecticides in southern Taiwan for several seasons. In the first study, DDT, dieldrin, fonofos, phorate, and carbofuran were applied at 5 kg/ha or at 10 kg/ha on 10 m × 10 m plots four times (on 9 December, 1974, 15 April, 1975, 30 October, and 26 April, 1976) and the residue levels in soil were monitored. They found that for all compounds the residue levels declined rapidly during May through August (summer months) for two successive years, while they increased during winter months. The summer months in Taiwan also represent the wet season, and, therefore, the seasonal variation may be a result of the combined effects of temperature and humidity. Also, it must be noted that in the case of carbofuran, phorate, and fonofos their degradation may have been aided by the alkalinity of the soil (pH 8.5–8.7). On the other hand, for these chemicals there was a trend of soil acclimatization leading to faster rate of disappearance of the parent compounds in the second season than at the initial time of application, indicating microbial participation in the process of pesticide degradation. An interesting observation was that under the application rates neither DDT nor dieldrin showed a tendency to accumulate over the test period. These authors have calculated that the time required for 50% disappearance of DDT and dieldrin during the hot and rainy season was 3.5 and 3 months respectively, which are considerably shorter times than those reported from temperate zones.

A similar pattern was observed for fenvalerate, a pyrethroid insecticide (Telekar *et al.*, 1983a) applied at 1 kg/ha five times in two years. After 2.5 years only 2% of the applied fenvalerate could be recovered. The pesticide residues declined rapidly during the hot and rainy months.

In the second study Telekar *et al.* (1983b) applied heptachlor, triazophos, and fenvalerate biweekly for a two-year period to small plots (5 × 4 m) with three different soil types. Residue patterns were monitored for three seasons. Again, the pattern of seasonal variations in residue levels was identical to the one observed in the first two studies. Comparing these data to those obtained in temperate regions these authors concluded that pesticides in general disappear faster in subtropical and tropical environments.

The above studies have clearly demonstrated that under their experimental conditions all pesticides tested degraded faster at high temperature and humidity. Care must be taken, however, not to generalize that pesticide degradation is always high at higher temperatures. In many parts of the world, microbial activities are known to decline during hot summer months and increase during cool spring and fall months. The tendency is particularly pronounced in areas where summer is the dry period of the year.

Microbial degradation of pollutants and pesticides in soil is influenced by many environmental factors. Thus there is a danger in simply assuming that a given compound would degrade faster under hot tropical conditions than it would under a cooler temperate environment. On the other hand, there are a few practical considerations which could help the reader to judge the conditions which favour or disfavour microbial degradation of organics in non-temperate climates.

First, microbial degradation of organics decreases dramatically below 10°C and practically ceases to operate at temperatures below 5°C in all environments. Second, there are much longer growing seasons in warmer climates than in colder areas. And third, high microbial activities are always associated with high moisture. Thus, microbial contributions to degradation of chemicals in the environment would be negligible in periods or areas where temperature stays below 5°C, or in places receiving very little water through rain, flooding, or irrigation. Therefore, at least in those extreme environments, microbial degradation of pollutants would be minimal.

In tropical zones the intensity of sunlight is higher, in addition to higher average temperatures throughout the year. Knowing that many pollutants are degraded by combinations of biotic and abiotic factors (*see* Section 3.1), these two factors would a priori promote overall reaction processes of pollutant degradation.

There are a number of examples indicating that the overall carbon utilization rates per year by soil microorganisms are higher in tropical zones compared to temperate zones. A good example is the case of coniferous forest litter, which tends to accumulate up to a few years in the extreme north, and to disappear in a few months in the south. As I indicated at the beginning of this chapter, some types of pollutant degradation are closely associated with overall microbial activities and/or biomass (e.g. wide-spectrum metabolism). Therefore, those types of metabolic activities are expected to be high in the south, when the rate of disappearance is considered on an annual basis (i.e. instead of comparing a given time period within a growing period of the year between north and south).

Another way to assess the overall differences between north and south in the rate of disappearance of pesticides is to examine dosages or frequency of pesticide application for comparable crops and pests. Certainly this approach is not foolproof as the variety of crops, even if the species is the same, as well as pest species or strains may be quite different in the south from those in the north. Also, such an approach does not take into account the role of abiotic factors as explained before. Nevertheless, taken together with other observations such an approach could be used as secondary evidence to indicate the north–south difference in this regard.

3.2.8 CONCLUSION

From the above discussion on relative microbial contribution on pesticide degradation between tropical and temperate zones, it has become evident that there is no simple straightforward trend that could be used to assess regional differences due to differences in temperature and associated climatic factors.

Only under extreme conditions, such as temperatures below 5°C, low humidity, lack of organic matter, etc., may a low rate of pesticide degradation be predicted. While there are some supporting factors (such as longer growing season, etc.) to the generally held belief that pesticides degrade faster in tropical zones than in temperate regions, one must be cautious in extending such circumstantial evidence to predicting pesticide degradation rates in any region. After all, high DDT residues are found in food chains and in man in India, where a short half-life of DDT had been predicted because of the country's predominantly tropical climate. Such an observation points to the need for cautious approaches in assessing pollutants' fates in the environment in non-temperate zones. Thus the only sensible approach is to assess residue characteristics and fate for each pollutant in each locality where pollution is suspected, until a reliable data base has been accumulated. One helpful aspect in this regard has been that microbial degradation pathways and metabolic products formed in tropical regions appear to be qualitatively identical or very similar to those found in temperate zones (Sethunathan *et al.*, 1982), suggesting involvement of similar metabolic activities among different zones and regions in this regard. Thus there is a good possibility of developing logical approaches to assessing basic mechanisms of pollutant degradation by microorganisms in each locality by applying basic knowledge accumulated in this field in the last few decades.

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