

## CHAPTER 13

# *Toxic Effects of Pollutants on Microorganisms*

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### 13.1. INTRODUCTION

Bacteria and fungi play a fundamental role in the biogeochemical cycles in nature. These microorganisms remineralize organic matter to carbon dioxide, water, and various inorganic salts. Because bacteria are ubiquitous, and capable of rapid growth when provided with nutrients and conditions favourable for metabolism and cell division, they are involved in catalysis and synthesis of organic matter in the aquatic and terrestrial environments. Many substances, such as lignin, cellulose, chitin, pectin, agar, hydrocarbons, phenols, and other organic chemicals, are degraded by microbial action. The rate of decomposition of organic compounds depends upon their chemical structure and complexity and upon environmental conditions.

The nitrogen cycle, including fixation of molecular nitrogen and denitrification, is mediated by microorganisms in the natural environment. Other biogeochemical cycles, including the sulphur, phosphorus, iron, and manganese cycles also depend primarily upon microbial activity. Transformation and mobilization of heavy metals, degradation of pesticides, herbicides, and other

man-made, allochthonous materials are left, ultimately to the microorganisms, for recycling. The toxic effects of pollutants on the autochthonous microbial populations, therefore, become of major significance in ecotoxicology.

### 13.2. MICROBIAL DEGRADATION OF POLLUTANTS

A wide variety of synthetic organic compounds contaminate the environment from chemical and industrial processes. In many instances, organic loads entering receiving waters add to the existing organic pools and cause perturbations in the natural degradation processes of the aquatic microbial community. Many chemicals employed in industrial processes are both refractory and toxic, and removal of these pollutants from the aquatic environment occurs primarily by microbial activities. Microbial degradation is dependent upon physical and chemical environmental variables, as well as on the toxicity of the chemical.

A list of the entire spectrum of industrial compounds entering the aquatic environment, either through chemical processes, accidental contamination, or as waste by-products, would fill volumes of books and catalogues. In a recent National Science Foundation report *ca.* 200 compounds were identified as being of national concern, because of their relative abundance as environmental contaminants or their relative toxicity. The most extensively studied of the chemical compounds are those representing the greatest threat to environmental health (Nelson and Van Duuren, 1975).

Physical and chemical factors may render a given compound more or less susceptible to microbial degradation. For example, irradiation in the visible and ultraviolet ranges can aid in the degradation of polymerized plastics and dechlorination of halogenated substrates and, perhaps, in the cleavage of alkylated biphenyls and fused aromatic ring systems. Photodegradation has also been implicated in the potential formation of chlorinated dibenzofurans from chlorinated biphenyls producing more toxic compounds of unknown biodegradative potential (Crosby and Moilanen, 1973).

Interaction of hydrophobic aquatic contaminants with dissolved organic substances and particulate matter can result in physical partitioning of the compound from the water column, bringing the susceptible substrate into closer association with those microorganisms capable of degrading the compounds. Such partitioning can also cause a concentration of the contaminant to toxic levels, thereby suppressing or retarding biodegradation or affecting the biological components of the ecosystem. Thus solubilization or partitioning of pollutants into dissolved organic phases can stimulate biodegradation, through availability of co-metabolizable substrates or inhibition of normal decomposition activity. Many biological compounds, such as lipids, proteins, nucleic acids, and amino acids concentrate or increase the solubility of chlorinated biphenyls and many polycyclic aromatic hydrocarbons.

Of the large variety of existing synthetic industrial chemicals, a number of classes of compounds have been studied (Table 13.1).

Table 13.1 Chemical Contaminants found in Aquatic Environments. (Reproduced by permission of John Wiley & Sons, Inc., from Colwell and Sayler, 1977)

Chemical class	Model compounds	Examples of degrading organisms	Reference
Phenolics	Phenol	<i>Pseudomonas putida</i>	Der Yang and Humphrey (1975)
Phenolics-halogenated	Pentachlorophenol	Soil bacteria	Kirsch and Etzel (1973)
Aromatic substrates			
monocyclic	Xylene	<i>Pseudomonas putida</i>	Gibson <i>et al.</i> (1974)
Monocyclic-halogenated	Fluorobenzoate	<i>Pseudomonas</i> spp.	Goldman (1972)
Bicyclic	Biphenyl	Soil bacteria	Lunt and Evans (1970)
Bicyclic-halogenated	PCB ('Aroclors')	<i>Pseudomonas</i> spp.	Sayler <i>et al.</i> (1977a)
Polycyclic	Benzo(a)pyrene	<i>Pseudomonas</i> spp.	Barnsley (1975)
Alkylated	Dibutylphthalate	<i>Brevibacterium</i> spp.	Engelhardt <i>et al.</i> (1975)
Chlorinated aliphatics	Trichloroethane	Marine bacteria	Jensen and Rosenberg (1975)
Glycols	Ethylene glycol	Aquatic bacteria	Evans and David (1974)
Petroleum	Hexadecane	Aquatic bacteria	Walker and Colwell (1974a)
	Mixed hydrocarbon substrate		



Petroleum offers a good example of the effects of pollutants on microorganisms since it is increasingly a problem in the world oceans, with toxic effects noted for some marine organisms. Since the earliest recorded oil spill in 1907 (Bourne, 1968), numerous reports have been published on oil pollution. These include 1,100 scientific manuscripts (Moulder and Varley, 1971), as well as popular publications (Marx, 1971; Nelson-Smith, 1973) and a number of symposia directed at the problem of oil pollution (Anonymous, 1969, 1970, 1971, 1973, 1975; Carthy and Arthur, 1968; Colwell, 1977; Hepple, 1971; Holmes and Dewitt, 1970; Hoult, 1969; D'Emidio, 1972; Ahearn and Meyers, 1973).

### 13.3. MICROBIAL ECOTOXICOLOGICAL EFFECTS OF PETROLEUM

The role of microorganisms in oil pollution has been emphasized by Davis (1967) and Sharpley (1966) in two textbooks on petroleum microbiology and several reviews on the subject have also been published (ZoBell, 1969; Friede *et al.*, 1972; Atlas and Bartha, 1973a; Crow *et al.*, 1974).

The community structure of the total viable microbial populations, the population shifts occurring as seasonal events or upon introduction of new metabolites, and the metabolic capabilities of the natural microbial flora are only beginning to be understood. The full extent of the effect of petroleum on both micro- and macrobiological communities remains to be clarified. Although it has been shown that components of petroleum persist in the marine environment (Hartung and Klingler, 1968; Holcomb, 1969; Horn *et al.*, 1970; Blumer, 1971; Blumer *et al.*, 1971, 1972; Blumer and Sass, 1972a,b), it is relatively easy to demonstrate in the laboratory that many marine bacteria, under optimal conditions, can remove selected fractions of oil, usually the *n*-alkanes, in a matter of days or weeks so that a certain percentage of oil by weight will disappear in a given period of time. However, it cannot be said that all the oil will disappear in a proportionate time, since the remaining fractions may be more refractory to microbial attack. In nature, conditions rarely are favourable for maximum biodegradation; hence, the rate of degradation can be slow and oil may persist in the marine environment for a long time. Floodgate (1972) surmises that some components of the 10 million tons of oil that found its way into the sea in World War II are still in the oceans. The significant questions of ecotoxicology of petroleum, then, are whether degradation demonstrated to occur in the laboratory will occur in the oceans; whether oil reaches depths of 1,000 m and, if so, the rates and degrees of oil degradation at depths greater than 1,000 m. It is not clearly understood whether concentrations of oil below some threshold level are degraded, since Jannasch (1970) noted that when the concentration of carbon in sea water was below a threshold level, bacterial growth was limited.

The qualitative and quantitative differences in hydrocarbon content of petroleum, influence its susceptibility to degradation, a major consideration in determining ecotoxicological effects of petroleum. Two crude oils and two refined

oils were examined for susceptibility to degradation by bacteria from an oil-contaminated environment, Baltimore Harbour in Chesapeake Bay, located on the southeastern Atlantic Ocean (Walker *et al.*, 1975a,b). The oils studied included South Louisiana crude oil, which contains a high percentage of saturates (56%), Kuwait crude oil, which contains 34% saturates and a significant amount (18%) of resins (pyridines, quinolines, carbazoles, sulphoxides, and amides), Fuel oil No. 2, a refined product composed of only saturates (61%) and aromatics (39%), and Fuel oil No. 6 (Bunker C) which can contain up to 45% asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins) and resins and forms 'tar balls' which may sink, if not dispersed. Degradation of the oils by bacteria was compared and it was found that after seven weeks, the quantity of the four oils remaining after exposure to bacteria from oil-contaminated sediment was approximately the same, indicating that, quantitatively, degradation of the four oils proceeded similarly. However, a comparison of the degradation of selected components of the four oils illustrated that there were, in fact, significant differences in susceptibility to degradation of fractions of the four oils, as was shown by column chromatography and computerized mass spectrometry (Walker *et al.*, 1975c).

Other investigators have recorded similar observations. Mulkins-Phillips and Stewart (1974b) compared the degradation of Venezuelan and Arabian crude oil by a *Nocardia* sp. The isolate degraded 77% and 13% of the *n*-alkanes in an unresolved fraction of Venezuelan crude oil, compared to 94% and 35%, respectively, of an Arabian crude oil. Atlas and Bartha (1973a) demonstrated that two paraffinic crudes, Sweden and Louisiana, were degraded similarly (70%) by mixed bacterial cultures grown in sea water. Jobson *et al.* (1972) compared growth of microbes on an inferior and high-grade crude oil. Microbes isolated on the inferior crude oil were more capable of using it than microbes isolated on high-grade crude oil. Westlake *et al.* (1974) reported that crude-oil composition affected the rate and extent of biodegradation, specifically of the *n*-saturate fraction, resins, and asphaltenes.

Microorganisms present in water and sediment samples collected from two areas in the same geographical location in Chesapeake Bay, an oil-polluted environment (Baltimore Harbour) and an oil-free environment (Eastern Bay), were compared for degradative capability on South Louisiana crude oil. Bacteria previously exposed to oil were found to be the most effective in degrading the crude oil.

Microorganisms present in samples collected from oil-polluted harbours located in different geographical areas were also compared for ability to degrade a South Louisiana crude oil. In this case, the cultures from Baltimore Harbour were found to be more effective in degrading the oil than cultures present in San Juan Harbour sediment. An increase in resins and asphaltenes occurring in the Baltimore Harbour cultures was noted, compared with one San Juan culture; these fractions may have been accumulated 'refractory' components or may have derived from microbial synthesis (Walker and Colwell, 1975a). Atlas and Bartha (1973b) reported that the accumulation of such components as fatty acids in flask cultures may prevent complete biodegradation of oil. Whether this occurs *in situ* is not known. It is of



interest that deep-ocean sediment samples yielded greater petroleum-degrading potential than those from Eastern Bay, an unpolluted site in Chesapeake Bay (Walker *et al.*, 1976b). However, Baltimore Harbour, the polluted site, yielded the greatest degradative potential.

The neritic and deep-ocean environments differ considerably, especially with respect to such variables as temperature and pressure, which can have an effect on hydrocarbon degradation. Experiments using hydrocarbons have been done (Schwarz *et al.*, 1974a,b, 1975) using  $^{14}\text{C}$ -labelled hydrocarbons and a mixed hydrocarbon substrate which revealed that at one atm pressure and  $4^\circ$ , the stationary phase for the *n*-hexadecane culture was reached within 4 weeks, but at 500 atm, the stationary phase was reached only after 32 weeks. Utilization of 94% of the hexadecane was accomplished after 8 weeks at 1 atm, but only after incubation for 40 weeks at 400 atm. Thus low temperatures and high pressures restrict the utilization of hexadecane by microorganisms and contribute to the persistence of these compounds in nature (Schwarz *et al.*, 1974a,b, 1975).

Limitation of nutrients, especially nitrates and phosphates, affects the degradation of petroleum occurring in the environment, contributing to the persistence of selected fractions of petroleum. Petroleum degradation is severely limited in the natural environment, unless nitrate and phosphate are added as a nutrient supplement (Atlas and Bartha, 1972b, 1973c).

Climatological conditions affect microbial degradation of petroleum and seasonal effects on the rate and extent of degradation have been observed. Two stations in Raritan Bay were monitored at five intervals between July 1971 and May 1972, and it was discovered that the highest counts of petroleum degraders were observed in July (Atlas and Bartha, 1973d). Increased numbers of petroleum degraders, however, were found at two time periods, December–February and June–July, in Chesapeake Bay (Colwell *et al.*, 1974). At each season larger numbers of petroleum degraders were observed in the oil-polluted environments of Chesapeake Bay than in the unpolluted environments. To determine if enrichment with psychrophilic petroleum degraders occurred during winter months, sediment bacteria from a polluted environment in Chesapeake Bay were sampled during January and September and these samples were tested for petroleum degradation at  $0^\circ$ . Differences were observed in the amount of oil degraded by microorganisms in those samples collected in January compared with samples collected in September, but they were not significant, suggesting that enrichment with psychrotrophic or psychrophilic petroleum degraders during winter months is not important.

The environmental variables involved in microbial degradation occurring in temperate and arctic zones are similar, except that in temperate or arctic zones, the specific conditions are established with much less variability. For example, oil polluting a permanently cold environment would be subjected to degradation by the pre-existing, psychrophilic or psychrotrophic microbial populations. The principal effects of low temperatures that must be considered are decreased volatilization and increased water solubility of the volatile hydrocarbons in

petroleum (Atlas and Bartha, 1972a; Walker and Colwell, 1974b; Colwell *et al.*, 1976b). Hence, the pollutant, oil in this case, may be more toxic in colder environments.

Bacteria (*Flavobacterium*, *Brevibacterium* and *Arthrobacter* spp.) have been reported to utilize 35–70% of the paraffinic crude oils, whereas fungi (*Penicillium* and *Cunninghamella* spp.) utilized 85–92%. Comparative studies by Walker *et al.* (1977a,b) demonstrated that yeasts (*Candida*) and fungi (*Penicillium*) degraded South Louisiana crude oil more extensively than bacteria (*Pseudomonas* and *Coryneforms*). Growth of bacteria in flask cultures on mixed hydrocarbon substrate was observed to precede growth of yeasts and fungi (Walker and Colwell, 1974a, 1975b). Also, oscillations were noted in the occurrence of several bacterial genera in mixed cultures growing on a mixed hydrocarbon substrate. Combinations of bacteria, yeasts, and filamentous fungi provided approximately twice as much degradation of the mixed hydrocarbon substrate, compared with each organism grown individually (Walker and Colwell, 1975b). Thus the mixed populations found in nature are more effective in degradation and detoxification than would be expected, based on pure-culture studies performed in the laboratory.

Reports by Atlas and Bartha (1973e) and Mulkins-Phillips and Stewart (1974a) describe effects of dispersants on the biodegradation of oil. Atlas and Bartha (1973e) found that dispersants increased the rate of mineralization, but did not affect the extent of biodegradation. Mulkins-Phillips and Stewart (1974a) examined four dispersants and found only one, 'Sugee' 2, that was associated with increased degradation of *n*-alkanes in crude oil, compared with crude oil alone.

ZoBell (1969), in summarizing work accomplished prior to 1969, reported rates of degradation of crude oils, lubricating oils, cutting oils, and oil wastes, ranging from 0.02 to 2.0 g/m<sup>2</sup>/day at 24° to 30°. Rates of oil biodegradation have been measured as g/m<sup>2</sup>/day, g/m<sup>3</sup>/yr, mg/day/bacterial cell or per cent oil removed after a known number of days. Thus it is often difficult to make comparisons of results based on the few studies that have been reported. Differences in experimental conditions provide additional problems when comparing the results of studies in which biodegradation rates of petroleum were calculated (Kinney *et al.*, 1969; Johnston, 1970; Kator *et al.*, 1971; Bridie and Bos, 1971; Robertson *et al.*, 1973). Although rates of biodegradation of petroleum in the environment have been estimated, often the experiments involved measuring the amount of oil at the beginning and termination of an experiment, assuming the rate to be linear. Furthermore, there are no studies of the fate of the petroleum components during biodegradation. In a recent study of the biodegradation of Louisiana crude oil (Walker *et al.*, 1976a), maximum degradation of the total residue was observed to occur during the logarithmic phase of bacterial multiplication (1.42 mg/day), with a levelling off at the stationary phase. Asphaltenes, resins, and aromatics increased after the stationary phase was reached, but saturates were degraded throughout the seven-week growth phase. Thus biodegradation of petroleum in the marine environment is influenced by a complex of ecological factors and it is an



oversimplification to cite a rate of biodegradation occurring in the ocean or other environments.

In general, microorganisms from an industrially polluted environment or from an oil-polluted harbour will carry out a more extensive degradation of a crude oil than microbes from an unpolluted environment. However, this is not an absolute rule, since deep-ocean sediment has been shown to contain microorganisms carrying out more extensive degradation of crude oil than, for example, sediment inocula collected in San Juan Harbour. As stated above, comparison of neritic and deep-sea microorganisms for ability to degrade petroleum under simulated *in situ* conditions demonstrates that, at least for pure hydrocarbons, deep-sea conditions clearly are associated with significantly slowed down degradation.

The fate of oil, after extensive exposure under various environmental conditions, has been examined by several investigators (Blumer *et al.*, 1972; Rashid, 1974). Paraffinic oils were found to persist as oils, not asphaltenes, for a 16-month period of monitoring. Changes in the oil were found to be due to weathering and microbial degradation. However, it is important to relate alterations in the structure of microbial populations with concomitant degradation of the oil substrate. This has been, in part, accomplished in the laboratory, but requires further study, particularly *in situ*.

From simulated *in situ* experiments, using flow-through sea-water tanks, designed to study biodegradation, it was discovered that oil-soluble phosphate and nitrate was required to enhance biodegradation of oil in sea water (Atlas and Bartha, 1973a). Thus sea water may not contain sufficient amounts of nitrate and phosphate to support significant degradation of oil, with the result that the oil, or fractions of the oil, will persist in the environment for long periods of time. In general, warmer temperatures appear to promote volatilization of low-boiling hydrocarbons, hence, faster utilization of metabolizable hydrocarbons. However, selection for psychrophilic petroleum degraders in colder climates may occur, but this point has not yet been resolved.

Crude oils and fuel oils have been found to have relatively little effect on heterotrophic microorganisms, in general, except in the case of yeasts, which tend to be slightly inhibited by fuel oil (Walker and Colwell, 1975a). However, crude oil and fuel oil have been found to limit the growth of specific groups of microorganisms, such as the lipolytic, proteolytic, and chitinolytic bacteria, with fuel oil observed to be more toxic than crude oil (Walker *et al.*, 1974b), despite the fact that both fuel oil and crude oil can support the growth of certain individual species of heterotrophic and cellulolytic bacteria (Walker *et al.*, 1975e).

Addition of petroleum hydrocarbons to water samples collected from an oil-free environment has been shown to limit the growth of the bacteria normally present in the water (Walker and Colwell, 1975a, 1977). However, addition of petroleum hydrocarbons to water collected from an oil-polluted environment promoted growth of bacteria already present in the water (Walker and Colwell, 1975a),



suggesting that selection for oil-resistant species had already occurred in the oil-polluted environment.

The positive chemotactic responses of motile marine bacteria have been shown to be reversed by the addition of sublethal concentrations of hydrocarbons, yet another ecotoxicological effect of oil in that it can interfere with microbial chemotaxis (Young and Mitchell, 1973). This is an important ecological phenomenon since microorganisms depend on chemotaxis for attachment to substrates for growth and nutrition.

With respect to the effects of petroleum, the available data reveal that there is a predictable pattern of events in the biodegradation of petroleum, irrespective of geographical location, with variations in time of occurrence in the steps in the degradative process and in sequential changes in the mixed microbial population structure, according to climate, season, etc. Furthermore, it is clear that no single microbial species will degrade any given oil completely. Bacteria are selective and many different bacterial species in mixed cultures are required for significant degradation. Furthermore, bacterial oxidation of hydrocarbons produces many intermediates which may be more toxic than the original hydrocarbon components. It is obvious that it is microorganisms that must be relied upon to carry out degradation of hydrocarbon decomposition products in nature. Unfortunately, the fraction of crude oil most readily and completely subject to attack by bacteria is the least toxic, e.g. the normal paraffins. Toxic aromatic hydrocarbons, especially the carcinogenic polynuclear aromatics, are degraded only very slowly. It must be added, also, that evidence has been presented showing synthesis of carcinogenic hydrocarbons by various species of bacteria (ZoBell, 1971).

An ecotoxicological effect of petroleum degradation is the severe requirement for oxygen during bacterial oil degradation (ZoBell, 1969). The complete oxidation of one gallon of crude oil requires all the dissolved oxygen in 320,000 gallons of air-saturated sea water. It can be concluded that where the oxygen content has been lowered by other kinds of pollution, either bacterial degradation of oil may cause additional ecological damage via oxygen depletion or not occur at all because of insufficient oxygen.

An aspect of the oil pollution problem which is only just beginning to be understood is the enrichment of heavy metals and organic compounds in the petroleum hydrocarbon pollutant (Duce *et al.*, 1972; Walker and Colwell, 1974c,d, 1976b).

No body of data exists concerning the effect of petroleum on autotrophic microbes, i.e. on those microorganisms upon which the biogeochemical cycles occurring in the sea depend. For example, the sulphur bacteria and nitrogen-fixing bacteria have not yet been examined to determine the effects of releases of petroleum hydrocarbons, both chronic and accidental, on these microorganisms.

Thus from all studies accomplished to date, it can be concluded that normal alkanes are the saturated hydrocarbons most readily susceptible to degradation.

Biodegradation of normal alkanes, however, always results in a residual base 'envelope', which can be detected using gas-liquid chromatography. These saturated hydrocarbons are 1- to 6-ring alkanes, as determined by using computerized low-resolution mass spectrometry (Walker *et al.*, 1975c). Also, microorganisms can produce polar *n*-pentane-insoluble components (asphaltenes) (Jobson *et al.*, 1972; Zajic *et al.*, 1974) and long-chain *n*-alkanes (Walker and Colwell, 1976a). Clearly, crude and fuel oils are degraded differently by microorganisms, with Bunker C fuel oil being degraded to a much lesser extent than South Louisiana crude oils. In fact, the aromatic fraction of Bunker C fuel oil is degraded only very slightly (Walker *et al.*, 1976b). For example, profiles of hydrocarbons in Baltimore Harbour sediment cores, i.e. presence in sediment samples according to depth, revealed that the more refractory compounds, including cycloparaffins, aromatics, and polynuclear aromatics are found in the deeper sediments (Walker *et al.*, 1974a, 1975c,d). Thus it is obvious that, although microorganisms are capable of degrading petroleum and petroleum by-products, there is a great deal of variability in the extent of degradation of the petroleum components, with the more 'refractory' components, such as aromatics and polynuclear aromatics, accumulating in the aquatic ecosystem.

#### 13.4. CARCINOGENICITY OF POLLUTANTS ASSOCIATED WITH MICROBIAL ACTIVITY

Little is known about the long-term hazards of oil contamination on aquatic ecosystems. However, one class of compounds in petroleum that is relatively resistant to microbial degradation is the polycyclic aromatic hydrocarbons, members of which are known to be carcinogenic (Miller and Miller, 1971). By-products of oil degradation, arising either from weathering or incomplete microbial mineralization, may also demonstrate carcinogenic activity. Several bacterial systems have been developed to detect compounds which are reactive with DNA and may cause mutation. Since most carcinogenic chemicals are also mutagens, these systems provide a quick and inexpensive means of screening large numbers of compounds or samples for potential carcinogenicity. The most widely used system is that developed by Ames and his colleagues (Ames *et al.*, 1975). About 85% of known carcinogens which have been tested by the Ames system have reacted positively as mutagens (McCann *et al.*, 1975). Many of these are promutagens, i.e. chemicals which are not mutagenic in themselves but which become so when acted upon by mammalian enzyme systems. They are detected in the bacteria system by inclusion in the assay of a mammalian liver microsome fraction. The mixed-function oxidases are implicated as the responsible enzymes in chemical conversion to carcinogenicity (Heidelberger, 1975). Since potent mutagens are reactive in the Ames systems in microgram quantities, the system is suitable for assaying for potential carcinogens in environmental samples.

To determine whether mutagenic metabolites were produced during bio-



degradation of oil by indigenous Chesapeake Bay organisms, Colgate Creek water and sediment samples were used to inoculate an inorganic Chesapeake salts' solution medium supplemented with oil as sole carbon and energy source (motor-oil medium, pH 7.0) (Voll *et al.*, 1977). Positive results in the Ames system were obtained with some samples of Colgate Creek water and sediment upon initial assay. Work is still in progress to test the Chesapeake Bay samples by the direct incorporation technique of Ames (Ames *et al.*, 1975) which is more sensitive in detecting low levels of mutagens or mutagens insoluble in water.

Although results to date are inconclusive, such systems do appear suitable for detection of potential carcinogens in samples collected for ecological analysis, but environmental samples present problems and difficulties not generally encountered with pure chemicals. Mutagenic substances may be present at concentrations below the level of detection of the assay, but significant concentration of test samples prior to assay can magnify levels of potentially toxic components. False negatives can arise with promutagens, if other components in the sample inhibit the liver homogenate activation system. With appropriate processing of environmental samples, these sources of error can be detected and eliminated. The most appropriate methods for processing and assaying samples from the environment and using bacterial assay systems will, no doubt, vary with the nature and source of the sample.

Degradation phenomena reported in laboratory studies of pure culture of microorganisms reflect only potential degradation that may occur in the natural environment. Physical-chemical properties of any chemical, environmental variables, and the concentration of the chemical, as well as the concentration and diversity of the microbial flora of a specific habitat, all are factors in the biodegradation process.

### 13.5. MICROBIAL EFFECTS ASSOCIATED WITH POLYCHLORINATED BIPHENYLS

Biodegradation of polychlorinated biphenyls has been studied by a number of investigators. Ahmed and Focht (1973a,b) demonstrated decomposition of mono- and dichlorobiphenyls by *Achromobacter* spp. isolated from sewage which resulted in the production of *p*-chlorobenzoic acid. In later studies (Alexander, 1975), respirometric data were presented that indicated degradation of PCBs to pentachlorobiphenyl, with the rate of degradation decreasing in direct proportion to increasing chlorine content of the PCB.

Kaiser and Wong (1974) investigated the pure culture degradation of 'Aroclor' 1254, a PCB mixture of biphenyl and other components, including a compound with seven chlorine residues per molecule. Results showed that significant degradation of the PCB could take place under appropriate environmental conditions. The bacterium was isolated by enrichment techniques from aquatic samples. Wong and Kaiser (1975) were able to demonstrate the degradation of

'Aroclor' 1221 (21% chlorine by weight) by mixed bacterial cultures in batch degradation studies.

Previously reported effects of PCB on microbial activity and growth responses have ranged from no effect on lipid biosynthesis in *E. coli* and *B. fragilis* (Greer *et al.*, 1974), to slight stimulation in cell growth in *E. coli* (Keil and Sandifer, 1972), to inhibition in the growth of selected estuarine bacteria (Bourquin, 1975).

Organisms capable of PCB metabolism have been recovered from estuarine and marine environments (Sayler *et al.*, 1977b); both PCB and PCB-degrading bacteria were found at the sites tested. However, no correlation between PCB levels and numbers of microorganisms degrading PCB were noted. High concentrations of 'Aroclor' 1254 were found to have no significant effect on the respiration of an estuarine *Pseudomonas* spp. capable of PCB degradation. However, stimulation of O<sub>2</sub> uptake was observed when 'Aroclor' 1254 was coated on diatomaceous earth and added to a pure culture at a concentration of 200 mg/l. Results of experiments carried out by several investigators indicate a significant potential for the removal of highly chlorinated biphenyls from estuarine water through biodegradation processes of a typical estuarine bacterial component.

The composite results from all the studies to date show that polychlorinated biphenyls are subject to microbial degradation, with the specific rate of degradation inversely related to the average chlorination of the PCB mixture and directly dependent upon the concentration of PCB. Furthermore, a wide variety of bacterial genera have demonstrated PCB degradation potential, although only preliminary results are available on the naturally occurring PCB degradation processes. There is no doubt that PCBs can induce changes in microbial population composition and activity.

### 13.6. EFFECTS OF COMBINATIONS OF POLLUTANTS

The biodegradative aspects of ecotoxicology at the microbial level indicate that many, if not most, of the pollutants entering the ecosystem can be degraded. However, there are effects of pollutants in combination, for example, mercury and oil; when these are combined, there is a suppressed degradation of oil and an enhanced toxicity because of the partitioning of the mercury in the oil phase (Walker and Colwell, 1976a). The toxic effect need not always be an enhancement since the toxic effect may be reduced if chelation occurs to a pollutant in a mixture. Clearly, given bodies of water and various mixtures of pollutants will yield an array of ecotoxicological effects.

### 13.7. TRANSFORMATIONS AND MOBILIZATION OF POLLUTANTS BY MICROBIAL ACTION

Implied in the biodegradative aspects of ecotoxicology described above are the transformations of pollutants into either more toxic forms or less toxic substances.



For example, under anaerobic conditions, sedimentary bacteria will methylate mercury, whereas under aerobic conditions, the microorganisms will produce the elemental form of mercury. The microorganisms act as mobilizing agents, in both cases, with mercury transported from sediment to water via microbial activity or mobilized through the food chain if the microorganisms concentrate mercury and are, in turn, fed upon by higher forms of the food chain (Sayler *et al.*, 1975; Sayler and Colwell, 1976; Colwell *et al.*, 1976a). Methylation of metals such as mercury, cadmium, and tin, by microorganisms results in either primary or secondary ecotoxicological effects, depending upon whether the pollutant is allochthonous to the environment or the transformation via microbial action yields the polluting substance.

Bioamplification of mercury levels in the oyster *Crassostrea virginica* has been shown to be mediated by bacteria (Sayler *et al.*, 1975), indicating the fundamental role of bacteria in mercury mobilization and accumulation of mercury at higher levels in food webs. Where there is a significant mercury-resistant bacterial population actively metabolizing as well as accumulating mercury compounds, their involvement in the bioaccumulation of mercury will be significant. This aspect of ecotoxicology at the microbial level, demonstrated for mercury and, no doubt, similar for other heavy metals, has yet to be fully appreciated.

Effects of metal ions on the microbial populations of estuaries has also been reported (Mills and Colwell, 1977), with significant effects on photosynthesis noted.

### 13.8. HEAVY METAL RESISTANCE, ANTIBIOTIC RESISTANCE, AND TRANSFER OF RESISTANCE FACTORS AMONG MICROORGANISMS

The severe impoverishment of the normal fauna by sewage sludge dumping in selected ocean sites is an effect that has been amply documented. Relatively unrecognized, however, is the effect on the microbial populations. In sewage sludges, relatively high concentrations of heavy metals are common. In such toxic environments, bacterial populations are selected by virtue of the rapid dissemination of resistance transfer factors (R plasmids). Antibiotic-resistant strains of *Enterobacteriaceae* reveal R plasmids which are highly transmissible between non-pathogenic and pathogenic donors and recipients by conjugation or transduction. The plasmids confer resistance to a wide spectrum of antibiotics and other antimicrobials, including heavy metals (Smith, 1967; Novick, 1969; Davies and Rownd, 1972; Summers and Silver, 1972; Koditschek and Guyre, 1974).

A large body of evidence has been accumulated showing a high incidence of R factor coliforms in both raw and treated sewage, river water, salt water, and in the New York Bight of the Atlantic Ocean (Feary *et al.*, 1972; Koditschek and Guyre, 1974).

The incidence of antibiotic-resistant bacteria in Chesapeake Bay was routinely monitored at selected stations over a 12-month period (Allen *et al.*, 1977). It was

found that in polluted areas of Chesapeake Bay, there was a significantly larger number of antibiotic-resistant bacteria that were, in addition, resistant to heavy metals. The data suggest that, indeed, in environments receiving a large influx of heavy metals, selection of heavy metal and antibiotic-resistant bacteria occurs. Transfer of plasmids appears to be significant in the transfer of resistance from the allochthonous to the autochthonous forms (Sizemore and Colwell, 1977; Guerry and Colwell, 1977).

### 13.9. MICROORGANISMS AS INDICATORS OF ENVIRONMENTAL POLLUTION

Bacteria have been employed as indicators of abnormal or atypical environmental conditions for many years. The most widely recognized indicators are the coliform group, used to monitor the presence and quantity of faecal pollution (Kabler *et al.*, 1964). Walker and Colwell (1973) showed that the number of petroleum-degrading microorganisms in water and sediments of Chesapeake Bay are related to the concentration of oil present. The concept of hydrocarbonoclastic microorganisms as indicators of hydrocarbons has been supported by several workers. A high correlation noted between ratios of hydrocarbonoclastic and total aerobic heterotrophic bacteria and levels of hydrocarbons in oil-rich salt-marsh sediments was reported by Hood *et al.* (1975), who also noted that the presence of hydrocarbons alters the relative abundance of the most predominant groups of the normal aerobic heterotrophic bacteria.

Similar relationships between mercury-resistant bacteria and concentration of mercury in the waters and sediments of Chesapeake Bay have been reported (Nelson and Colwell, 1975). Natural aquatic environments have measurable, reasonably definable microbial biota. Thus fluctuations in the environment result in a change in the delicate balance of the microbial community structure. In general, a single indicator group as the faecal coliforms, has been used to detect changes arising from pollution. If several indicator groups were used in combination, the effects of environmental changes could be more accurately measured. For example, the presence of crude oil has been shown to decrease the relative concentrations of cellulolytic bacteria in salt-marsh ecosystems (Crow *et al.*, 1975) and in Chesapeake Bay (Walker *et al.*, 1975e). Especially attractive is the potential for early warning of environmental change since microbiological responses are rapid and can be detected within hours or days. The microbial potential, perhaps measured as a community structure index, or other mathematical formulation, should be more fully investigated as an ecotoxicological yardstick of health. Clearly, the microbial aspects of ecotoxicology should be explored since here lies, indeed, a fertile ground for discovery and application in environmental pollution.



## 13.10. REFERENCES

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