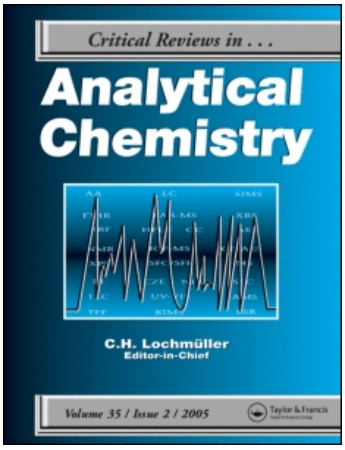

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Pathways and Analytical Tools in Degradation Studies of Organic Pollutants

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Pathways and Analytical Tools in Degradation Studies of Organic Pollutants

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All chemical compounds may undergo a variety of processes resulting from chemical, biological, or photochemical reactions. Depending on the environmental compartment in which organic compounds are present (e.g., soil, benthic sediments, surfacewaters, and groundwaters), they can undergo slow changes resulting from different chemical, physical, biological, or photochemical processes. In this study, different degradation pathways for selected persistent organic pollutants under varying conditions are presented. The problem of intermediate products that form during the degradation of substances, the toxicity of substances that are the products of organic compounds degradation, and the ways to identify such substances have been discussed.

Keywords organic pollutants, degradation, photodegradation, biodegradation, kinetics, analytical tools

INTRODUCTION

Persistent Organic Pollutants (POPs) mainly emitted to the environment from anthropogenic sources, are in the most cases lipophilic and can be characterized as toxic, stable, and having a tendency to bioaccumulate and biomagnify (1, 2). Depending on the environmental compartment in which compounds like polycyclic aromatic hydrocarbons (PAHs), chlorophenols, polychlorinated biphenyls (PCB), some pesticides and dioxins (PCDD and PCDF) are present (e.g., soil, benthic sediments, surfacewaters, and groundwaters), they can undergo slow changes resulting from different chemical, physical, biological, or photochemical processes. Degradation processes occur at various rates that depend on the type of compound and matrix in which it is present, as well as on environmental factors characteristic for a given matrix. The newly formed products of degradation may become a bigger or lesser harmful for the environment.

The degradation processes of environmental pollutants can be considered from one side in the context of using these processes in natural and man-designed ways, which are introduced to technological practice to clean up the particular environmental compartments (remediation). Then, estimation of degradability of compounds, which reach the environment in significant and even negligible quantities, is necessary in asserting the entire hazard associated with their use. On the other hand, degradation can be studied in the context of researching the influence of this process on changes in the composition of environmental samples. In this case, the degradation of analytes in a sample before the final measurements can make the interpretation of the obtained analytical information significantly more difficult. Furthermore, degradation during sample storage step, which frequently takes place, should be minimize as significantly as unlikely.

In all above cases, it is considered necessary to know the processes, so that their effectiveness can be controlled (e.g., remedial technologies) or the influence of degradation on analytical results can be eliminated. In addition, the knowledge of degradation pathways for particular compounds can facilitate the assessment of environmental pollution with POPs, based on the presence of degradation products.

The degradation of organic compounds in the environment can take place with the help of microorganisms and enzymes, under aerobic and anaerobic conditions. As a result, not only a modification of particular functional groups may occur, but, in the majority of cases, a degradation of basic structure of the compound takes place that will lead to complete decomposition to carbon dioxide, water, and inorganic salts (3). Presentation of

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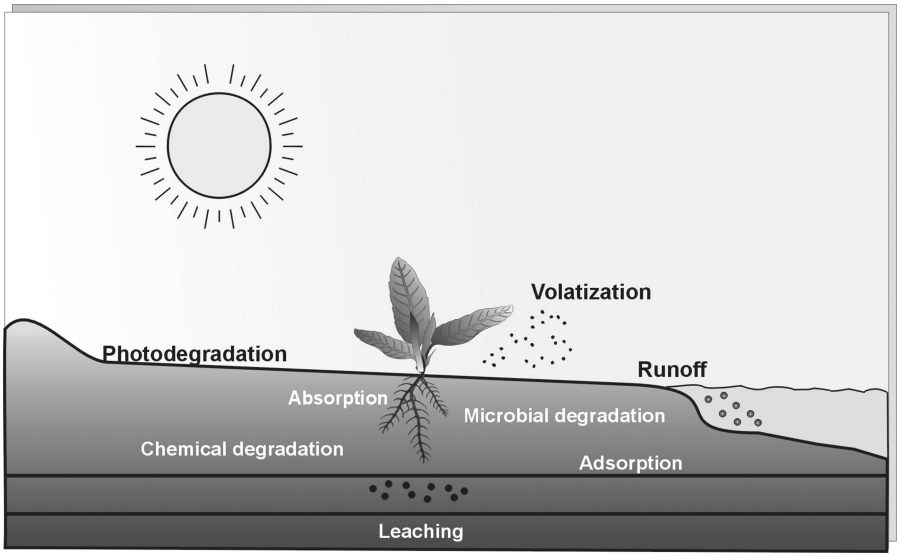

FIG. 1. Main processes to which persistent organic compounds are subjected in the environment.

degradation processes going on in the environment is shown in Figure 1.

Considering the analytical aspects, the knowledge of how stable a compound is in the particular environmental compartments, as well as of the degradation products, has great value for the validity and reliability of analytical results. It is important to ensure that the obtained measurements reflect the compound concentration in the investigated matrix at the moment of sample collection.

DEGRADATION OF THE SELECTED ORGANIC COMPOUNDS IN THE ENVIRONMENT; PATHWAYS AND ANALYTICAL PROBLEMS

Degradation processes are extremely important in the context of the level of pollution in particular environmental compartments. Extensive research has been conducted in many scientific centres to elucidate the influence of various factors on the effectiveness of degradation processes. Most of the remedial technologies have been based on the application of appropriate degradation pathways.

Bioremediation

Bioremediation is an effective and economical way to remove organic pollutants from water as well as soil, by using microorganisms and in the presence of fungi that stimulate degradation processes. The most frequently removed organic compounds are crude oil products, polycyclic aromatic hydrocarbons, and organo-chlorine derivatives. The appropriate bioremediation takes place when pollutants are degraded into nonhazardous, natural substances, and that, in turn, lowers costs and eliminates the need for treating the contaminated soil at the dumpsite. Bioremediation occurs in a humid as well as in a dry environment. The basic steps of removal are as follows: the introduction of an aerating system or the equivalents of oxygen; the introduction of nutritive medium and bacterial strain under controlled temperature and humidity. The unique feature of this system is that the contaminated soil may be localized in the environment where there are no conditions to conduct excavation or where this type of work may be noneconomical, e.g., roads or buildings. A very positive aspect of bioremediation is the elimination of costs associated with the disposal of pollutants. Moreover, under many circumstances bioremediation is the only most economical and available technology.

Besides bioremediation (here meaning the use of natural microbial flora in soil), there also is bioaugmentation, which is a process of introducing into soil-selected organisms capable of decomposing certain contaminants (4, 5). In recent years, it has been found that cultivating plants might also play a significant role in bioremediation processes (the so-called, phytoremediation). The natural conditions are the main constraint for phytoremediation, particularly the high, above the tolerance level of plants, content of pollutants in soil. Unfortunately, the information on phytotoxic impact and hazardous for plants concentrations of PAHs in soils is almost entirely lacking.

A variety of bacterial species and radiolarians take part in chemical reactions occurring in organic compounds, particularly in crude oil derivatives. The typical strain are *Pseudomonas*, *Arthobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Acitenobacter*, *Micrococcus*, *Nocardia*, and *Mycobacterium*. In addition, filamentous fungi, mainly from the genera *Fusarium*, *Aspergillus*, and *Penicillium* display degradation capabilities (6).

Biological treatment is a safe and natural way to utilize the polluted soil or water because bacteria. When once applied, remove the contamination from soil and water under every climatic regime, in different weather conditions and in various geological

formations; a low oxygen and nutritive medium demand is a strong positive feature of this process.

During microbial decomposition of pentachlorophenol (PCP) described by McGrath and Singleton (7), over 30 products form, of which some are more toxic than the parent compound. The changes occurring in PCP in soil in the presence of the bacterial species *Bacillus megaterium,* and after the previous addition of commonly used fungal strain *Phanerochaete chrysosporium*, have been investigated. After a 6-week incubation at 25°C, the PCP concentration decreased over 100 times; however, the acceleration of bioremediation due to addition of *P. chrysosporium* was not observed. The pentachlorophenol degradation was observed in the soil sample contaminated with PCP as well as in the sample containing PCP and *P. chrysosporium.* During the PCP degradation in soil, a number of toxic products were formed (among others, 3,4,5-trichlorophenol and 2,3,4,5-tetrachlorophenol) that, undoubtedly, would have undergone decomposition if the incubation had lasted longer.

Gonzalez and Wei-Shou (8) also studied the degradation of pentachlorophenol by using bacterial cells from the genus *Flavobacterium*. The results of an experiment showed that, in accordance with the first-order kinetics, some bacteria die during the period of adaptation while the rest remain capable of growing and decomposing PCP. The schematic degradation pathway of PCP by *Flavobacterium* is presented in Figure 2. It has been also observed that the level of PCP concentration influences the adaptation period of microorganisms—the period is longer for higher PCP concentrations (8).

The Degradation of Compounds During the Preparation of Samples for Analysis

Degradation processes occurring in the collected environmental samples, i.e., during sample collection and preliminary handling and storage, are definitely undesirable because they can significantly influence the concentrations of analytes and the overall stability of sample composition. This, in the end, may result in misinformation of analytical data that are of the high importance concerning presence and content of particular analytes in the investigated environmental compartments. Therefore, it is necessary to know precisely the processes undergoing with particular analytes present in a sample, so preventive action can be taken in order to fulfil the requirement of sample representativeness in reference to the object of investigations, until the beginning of the analysis proper. Quite frequently the collected samples have to be stored for a period of time before the analysis (for example because of time and equipment constraints in the laboratory). Therefore, the information on a maximum sample storage time, i.e., a storage time before the change in sample composition occurs, is of the utmost importance. Otherwise, the results of a conducted sample analysis will reflect not the concentrations of analytes, but rather the concentrations of completely different components, which were not present in a sample during its collection. Because of the fact that in the laboratory, under the influence of temperature and light, degradation occurs much faster than in the natural environment, a particular attention has to be paid to these processes.

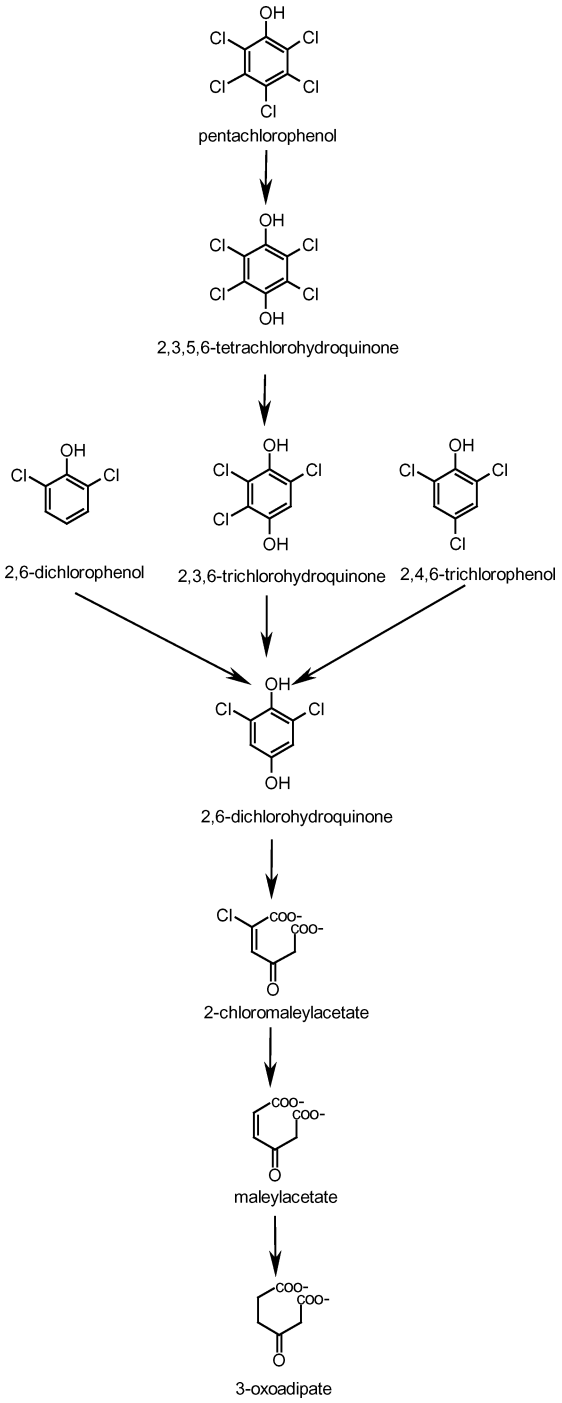

FIG. 2. Biodegradation pathway of PCP by *Flavobacterium* (9).

Undesirable changes in the sample composition can be minimized by choosing a proper method of sample preservation. However, even after sample preservation and storage under proper conditions, its stability is not completely certain. It is impossible to assure stable concentrations of all sample components at the same time.

Degradation processes occurring in the collected environmental samples, i.e., during sample collection and preliminary handling and storage, are definitely undesirable because they can significantly influence the concentrations of analytes and the overall stability of sample composition. Therefore, a preventive action has to be taken in order to fulfil the requirement of sample representativeness and the information on a maximum sample storage time, i.e., a storage time before the change in sample composition occurs, is of the utmost importance.

Although choosing a proper method of sample preservation can minimize undesirable changes in the sample composition; however, even after sample preservation and storage under proper conditions, its stability is not completely certain. It is impossible to assure stable concentrations of all sample components at the same time. Nevertheless, there are some papers that deal with methods for sample preservation presented currently (10–18).

THE KINETICS OF DEGRADATION PROCESSES

Even though POPs exhibit high persistency, meaning here the ability to stay unchanged in the environment for long time, it is well known that after some time, they start to degrade. According to this knowledge, based on the literature assessment of degradation processes, degradation reactions are typically described by first-order equations (19–25).

The first-order degradation kinetics may be expressed as follows (26, 27):

$$dC/dt = -k_1C \quad [1]$$

where C represents the concentration of a degraded compound at the time t; k_1 is the first-order rate constant. In practice, the first-order rate constant often is replaced by a half-life (H) and the degradation rate is expressed as follows (28):

$$dC/dt = -(0.6933/H)C \quad [2]$$

where $H = 0.693/k_1$.

If half-life (H) remains constant in a degradation process, the residual concentration C(t), may be expressed as an exponential function of time t according to the following equation:

$$C(t) = C_0e^{-(0.693/H)t} \quad [3]$$

where C_0 is the initial concentration. From the expression above, we see that the logarithm of the concentration is a linear function of time and therefore can be written as follows:

$$\ln(C(t)) = \ln(C_0) - (0.693/H)t \quad [4]$$

According to Martins et al. (23), degradation of rimsulfuron in soil and water followed first-order kinetics. Data from El-Dib and Abou-Waly (21) concerning biodegradation of such herbicides, as gardoprim, igran, dicuran, and patoran by natural microflora of river water also followed first-order kinetics. As stated by Klečka et al. (24) the biodegradation of bisphenol A (2,2-(4,4-dihydroxydiphenyl)propane), assessed in surface waters from several different rivers appeared to follow first-order kinetics (the slope of the biodegradation curves and corresponding pseudo-first-order rate constants were relatively uniform over the range of initial test chemical concentrations, μg/L level). Data from Yuan et al. (25) reported that biodegradation of PAH in river sediment also followed first-order kinetics. Sakks and coworkers (29) studied the aqueous photodegradation of dichlofluanid. It was found that the photodegradation proceed via a first-order reaction. As indicated by Penuela and Barceló (30) photodegradation of one of the organochlorine pesticides, such as chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), is an effective method to remove these pesticides from the aquatic environment. In this case, all photodegradation reactions followed first-order kinetics as well.

The time, which is needed for half of the amount of chemical to be removed from the environment, defined as half-life time, is used to compare the persistence of different chemicals with each other or with that of the standard. Half-life is sometimes defined as the time required for half the amount of substance to be completely degraded and released as carbon dioxide. Usually, the half-life time of a substance measured by the latter basis is longer than that based on deactivation only. This is especially true if toxic or nontoxic metabolites accumulate in the soil during the degradation. Half-life time values in subsoil and in groundwater are usually much larger. Thus, as compounds are leached to lower depths, their persistency increases.

One of the potentially adverse consequences of persistence is a buildup of environmental concentrations. This is illustrated in Figure 3, which shows how environmental concentrations change for chemicals with different half-life times. The maximum environmental concentration reached for each substance depends on its half-life time. Substances with short half-life times (1 to 100 time units) soon reach a balance between emission and removal at a characteristic ("steady-state") environmental concentration. Once emissions stop, the environmental concentration drops back toward background levels. On the other hand, for substances with long half-life times, the environmental concentration keeps increasing. Even when emissions stop, the concentrations fall very slowly.

In practice, emissions and discharges of a chemical will be different to each environmental compartment. Some chemicals

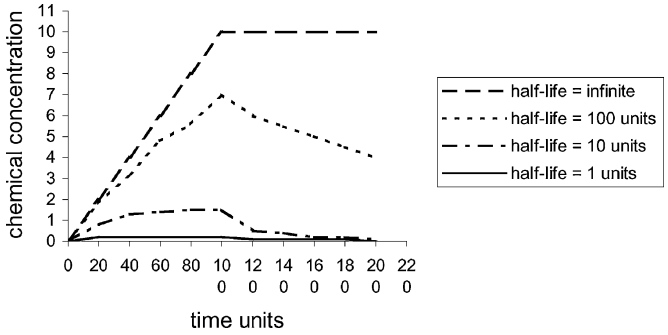

FIG. 3. Environmental concentrations of chemicals with various half-life times (31).

may be mostly emitted to air while others may be mostly discharged to water. Similarly, in each of these environmental compartments, the removal processes and different rates of removal will vary, depending on the characteristics of the chemical. The properties of the chemical (e.g., its solubility in water, its volatility, and polarity) will determine its tendency to move from one environmental compartment (e.g., water) to another (e.g., soil, sediment, or air) and will influence its susceptibility to biological and chemical breakdown (31).

The actual rate of disappearance of a chemical from the environment will depend on the processes available for removing it. Typical removal processes are: biological breakdown, (e.g., bacterial degradation in soil or sediment); chemical (abiotic) breakdown (e.g., hydrolysis in soil, sediment, or water); and transfer to a different environmental compartment, (e.g., volatilisation and/or evaporation from water into the air).

In practice, the different processes for each compartment and their relative rates must be considered to assess the overall persistence of the chemical in the environment. These processes and their rates depend in turn on the nature of the environment as well as the native properties of the chemical. For example, both chemical and biological breakdown rates will depend on the temperature, moisture, and pH (acidity) of the environment. Biological breakdown will also depend on the number and types of bacteria and other microorganisms present. Details on the degradation pathways of pollutants are discussed further.

DEGRADATION PROCESSES OF ORGANIC POLLUTANTS IN THE ENVIRONMENT

Depending on the environmental compartment in which organic compounds are present, they can undergo slow changes resulting from chemical, biological, or photochemical reactions. Photodegradation and biodegradation are major degradation processes, which can naturally clean up the environment.

Photodegradation, as a chemical reaction that occurs under the influence of photons or light, may take place in the atmosphere and on the surface of either water or soil, where as it does not occur in benthic sediments and deep layers of soil. The intensity of UV radiation depends on many factors, among others, time of the year, time of day, latitude, height above the sea level, air density, cloud cover, or the size of the ozone hole (32).

Biodegradation is a multistep process that is taking place in the presence of a number of microorganisms that often act synergistically. The range and rate of biodegradation processes depend on several factors such as the composition and activity of bacterial flora, the properties and "age" of a pollutant, the content of nutritive ingredients and physicochemical properties of medium in which the reactions occur (19, 33, 34).

Photodegradation

The sunlight reaching the Earth has a wavelength of over 286.3 nm (35). The majority of UV rays are absorbed in the surface water layer (down to a 2 m depth); however, they can reach deeper depths under the condition that light penetrates there. When discussing photochemical reactions it has to be pointed out that there are two types of processes, namely, a direct photolysis and an indirect photolysis (36).

Direct photolysis is a process in which molecules get excited by the absorption of a photon, and that results in a chemical reaction, usually oxidation. Photolytic potential depends mainly on the degree of overlap between the UV/VIS absorption spectrum of the compound and the emission spectrum of the beam of sunlight in the range 290–750 nm. The direct effects of UV irradiation include transformation of organic compounds into other substances, breaking of chemical bonds, or even complete degradation of organic substances. In addition, UV radiation causes the dissociation of oxidizing compounds and formation of highly reactive radicals that are capable of degrading organic pollutants (37).

Indirect photolysis of substances occurs through a reaction with OH-radicals, ozone, or NO_3; these three chemicals are considered the most important photo-oxidizing agents present in the atmosphere. The number of reactive radicals, such as ·OH or free oxygen, changes during a 24-hour cycle, starting with zero radicals at night and reaching a maximum at noon. Over 90% of organic compounds occurring in the gaseous phase of troposphere undergo transformations resulting from the reactions with OH-radicals (38–40).

The mechanism of radical formation through photolysis consists of several stages. Firstly absorption of a quantum of energy (i.e., photon) by a molecule takes place. Then, breakage of chemical bonds within the molecule occurs as a result of irradiation of sufficient energy (the energy increases as the wavelength decreases; $E = hc/\lambda$). This step is followed by formation of very reactive intermediate forms of radicals, i.e., ·OH, ·OOH.

Photodegradation of Organic Compounds in Aqueous Media

The phototransformation of a compound in surface water may result from light absorption by the pollutant itself (direct photolysis) or may be photoinduced by the dissolved natural organic matter or nitrate ions present in water, as these chromophores are known to photoproduce reactive species (indirect photolysis). In aquatic environment, the processes of direct and indirect photolysis occur concurrently. The presence of microorganisms, algae, or humic substances accelerates photochemical reactions because these components are capable of absorbing sunlight. Some nonionic organic compounds, particularly pesticides, undergo photodegradation much faster in the presence of photosynthesising microorganisms (41). Based on the results obtained by Zepp and Schlotzhauer (40), it has been concluded that the majority of PAHs get photolyzed much faster in the presence of algae.

According to Wu et al. (36) phenol degrades through direct photolysis, thermal disassociation, and the reaction with

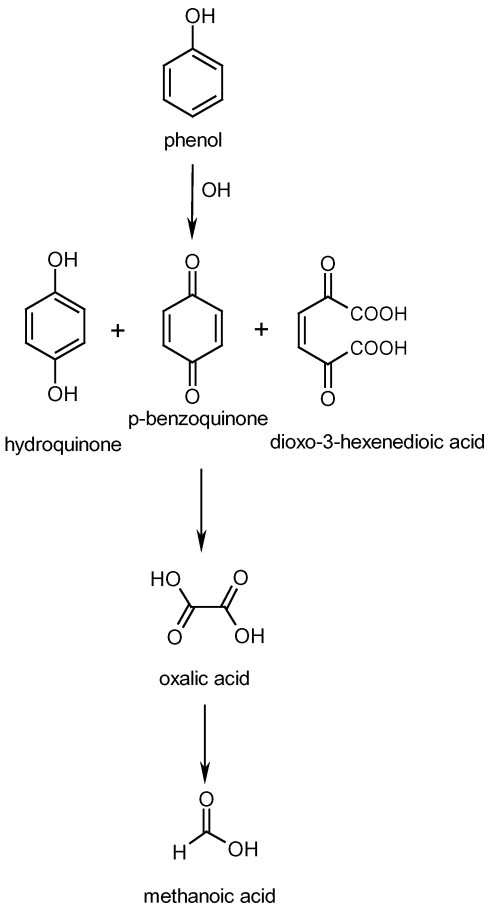

FIG. 4. Schematic presentation of phenol degradation in aqueous solutions (42).

hydroxyl radicals ·OH. The rate of phenol degradation increases with a decreasing value of pH and an increasing oxygen concentration in water. The presence of intermediate products of the reaction (i.e., hydroquinone, p-benzoquinone, and catechol) proves that ·OH takes part in the process of phenol degradation according to the scheme presented in Figure 4.

The investigations of Jongki et al. (43) showed that the main photodegradation products of pentachlorophenol, a compound commonly used as herbicide, insecticide, and for treating wood, are substances that form during the oxidation by hydroxyl radicals, as well as products originating from the reduction of chlorine atoms in ortho and para configurations. Tetra-, tri-, and dichlorophenols are typical products of successive dechlorination of pentachlorophenol. During the reactions of dehydroxylation, chlorination, or dechlorination, hexachlorobenzene and pentachlorobenzene are formed, and these, in turn, get oxidised to hexachloroquinone and tetrachlorocatechol. Further oxidation leads to the formation of 1,2,4-trihydroxytrichlorobenzene. Such degradation products are also formed when the reactions take place in organic solvents, i.e., acetone. However, the products are absent in the presence of oxidizers, such as TiO_2 or H_2O_2. This proves that depending on photolytic conditions, various degradation products can originate. Besides the main photolytic products, the presence of polychlorinated diphenylethers (PCDPEs) and polychlorinated dibenzo-*p*-dioxin (PCDDs) have also been observed.

Photolysis plays a significant role in the process of transformation and decomposition of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in aqueous solutions (44, 45). These compounds are characterized by high persistence, toxicity, and a tendency to bioaccumulate in live organisms. The rate of photochemical reactions depends on the number of chlorine atoms in a compound; therefore, the more C-Cl bonds, the slower the rate. MeeKuyung and O'Keefe (44) investigated degradation of selected PCDDs and PCDFs during exposition to the laboratory UV light (300 nm) and to sunlight in situ. The obtained results showed that, for the wavelength $\lambda = 300$ nm, the analytes from the PCDF family degrade faster than PCDDs. The rate of photolytic degradation of PCDF analytes in clean water is higher than in an aqueous medium containing acetonitrile, while in the case of PCDDs, the opposite is true. The underlying cause of this phenomenon most likely lies in the structure and polarizability of the compounds. The compounds from the PCDF family contain in their structure only one oxygen atom, and those result in a better polarizability as compared to PCDDs. They are also characterized by a higher reactivity in clean water than in the less polar aqueous medium containing acetonitrile.

Photolysis is a major abiotic degradation process (chemical oxidation and photooxidation) for many PAHs in the aquatic environment. Generally, compounds with higher molecular weight and more condensed aromatic rings have a higher rate of photolysis than smaller and less condensed ones. In the case of phenanthrene and anthracene, the molecular structure has been shown to be of importance, with typically lower photoreactivity in nonlinear molecules (46, 47). According to Lehto et al. (48), the photodegradation of PAHs, which is assumed to be a preliminary process after which the microbial decomposition of compounds occurs, causes the formation of partially oxidized, intermediate compounds that are more susceptible to biodegradation than the parent compounds. Quinones are intermediates in the environmental oxidation of PAHs. Anthracene readily oxidized with formation 9,10-anthracenedione (anthraquinone) as the primary product (49). Photolysis of benzo[a]anthracene and benzo[a]pyrene in water is slowed down considerably when amended with humic acids (50). The proposed explanation for this observation was that humic substances caused a quenching or scavenging of PAH-excited states, free radicals, or other excited-state complexes that participate in the photochemical reaction (51).

The principal degradation pathways for pesticides involve photolysis, hydrolysis, dehalogenation, and oxidation. Photochemical degradation is one of the major transformation processes and one of the factors controlling the fate of pesticides in the environment. Photodegradation can destroy pesticides on foliage, on the soil surface, and even in the air. Considering the chemical structure, pesticides have been classified into organic

and inorganic compounds. Factors that influence pesticide photodegradation include the intensity of the sunlight, properties of the application site, the application method, and the properties of the pesticide.

Vialaton and coworkers (52) studied the photolysis of propiconazole in pure water, in water containing humic substances and in natural water. Propiconazole was photodegraded in solar light. The phototransformation was faster by about 30% in natural water than in pure water.

Organic pesticides comprise, among others, phospho- and chloroorganic compounds, the derivatives of phenoxyacetic acid, triazine, and many others (53, 54); they are present in all the environmental compartments. When washed out from soil, they get transferred not only to the rivers, lakes, and oceans, but also to groundwaters. The photochemical pathways of fungicides (e.g., N-dichlorofluoromethylthio-N,N'-dimethyl-N-phenylsulfamide) in the samples of natural waters, i.e., seawater, riverine, and lake water, and in distilled water have been investigated by Sakkas et al. (55). Based on the experiment conducted, it has been concluded that the degradation of dichlofluanid is slower in natural water than in distilled water. It was possible to obtain the following ranking of degradation rates for different water bodies: lake water < riverine water < seawater < distilled water. Such paradigm shows a strong interrelation between the level of photodegradation and the presence and concentration of dissolved organic matter in particular matrices. With an increasing concentration of total organic carbon (TOC) in natural waters, the rate of photodegradation decreases. Microorganisms and sediment particles suspended in water cause the dispersion of light and, therefore, can remain a certain barrier for the light penetrating deeper water layers. The photolysis in samples exposed to the light under laboratory conditions was faster than in those subjected to the direct sunlight. This resulted from the fact that the sunlight intensity varied in dependency on a time of the day and atmospheric conditions, while the radiation intensity in the laboratory was constant during the entire experiment. Nevertheless, in all cases the presence of new compounds, possibly the products of degradation, was observed. Four of the compounds have been identified as aniline, dimercaptosuccinic acid (DMSA), dichlorofluoromethane, and *n*-dichlorofluoromethylthion-aniline (Figure 5).

Most of the photolysis studies (56, 57) have used natural sunlight, so it is unclear what wavelength range of light actually contributes to the photolytic reaction. Hirahara and coworkers (58) compared the photolysis of fenthion and disulfoton (organophosphorus pesticides) in liquid and solid phases with different sources of visible light and ultraviolet to estimate the extent of their photodegradation. They concluded that an UV wavelength of light is capable of causing the photolysis of fenthion and disulfoton. UVB is primary responsible for the photodegradation in the environment, and that fenthion is more readily degraded than disulfoton.

The photo-oxidation rate changes depending on the solvent. The rate constant obtained for fluorene decreased in the order of

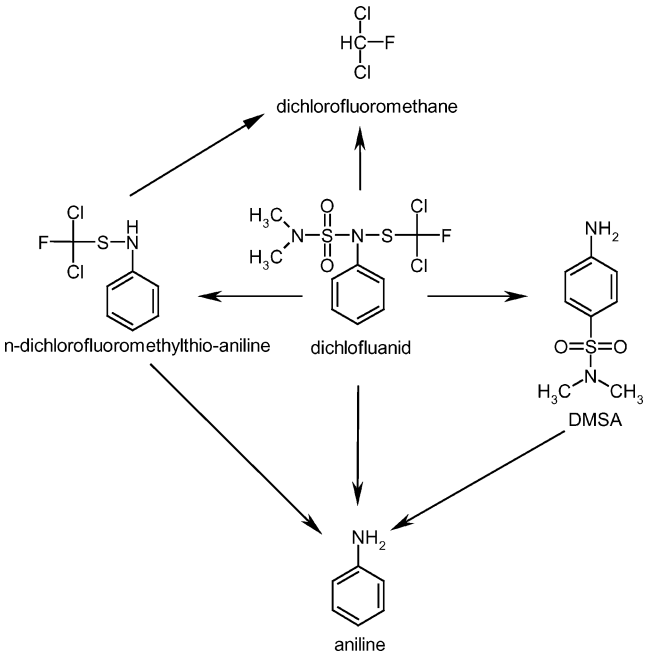

FIG. 5. Photodegradation pathway of dichlofluanid in aqueous solution (55).

dichloromethane > acetonitrile > methanol > acetonitrile/water (70/30) > acetonitrile/water (50/50); nonpolar solvents generally gave higher values than the polar ones. This is mainly due to difference in the solubility of oxygen (59).

Recently, the review, which accounts for the current knowledge about the distribution, accumulation, and chemical/photochemical transformations of persistent, bioaccumulative, and toxic compounds in waterice, especially in the connection with polar regions and atmospheric cloud particles has been published by Klán and Holoubek (60). According to the authors, ice photochemistry might play an important role in the chemical transformations in cold ecosystems and in the upper atmosphere, particularly now when the ozone layer is partially depleted. Photoreactions resembling liquid phase photochemistry are expected in a quasi-liquid water layer on the surface snow (ice) grains at higher (subzero) temperatures, especially when the organic substances are more hydrophilic (i.e., water soluble). Photochemical degradation of 4-nitrophenol in ice pellets is a good example as presented by Dubowski and Hoffmann (61). They found similar photoproducts, hydroquinone, benzquinone, 4-nitrosophenol, etc., as known from 4-nitrophenol photolysis in aqueous solutions. The results suggest a similar mechanism for the decomposition in both liquid and solid states (Figure 6).

The possibility that ice photochemistry can lead to the formation of new and unexpected organic compounds of high environmental risk, supported by some laboratory experiments, brings a new insight into the problem of what will be the result of global warming processes if ice melts.

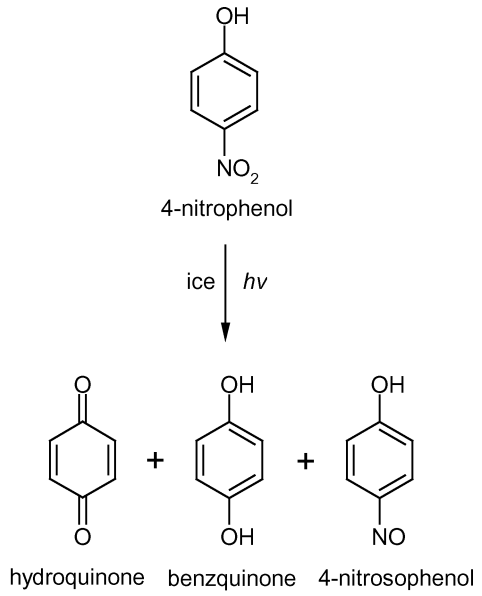

FIG. 6. Photochemical degradation of 4-nitrophenol in ice pellets.

Photodegradation of Organic Compounds in Soil

The concentrations of hazardous substances in water or atmosphere decrease relatively fast as a result of mixing and dilution, while many organic pollutants tend to accumulate in soil. Nonionic and nonpolar hazardous organic compounds tend to adsorb by humic substances. They accumulate in the surface layer of soil because most of the organic matter is present there. An organic compound can undergo photolysis after adsorption by colloidal substances in soil, and also when it is present in the surface layer of soil exposed to light (62). Therefore, photochemical reactions in soil are only, and exclusively, restricted to its surface; they extend to the maximum depth of 1 mm (41). Nonbiological degradation, i.e., photodegradation, occurs concurrently with the reaction catalyzed by microorganisms.

Photolysis may occur on soil surfaces although judgment should be practiced if rate constant values from this data are used. For example, laboratory studies measuring the photolytic degradation of DDT in soil reported a half-life of 125 days (63); however, field studies in soil showed that this compound had substantially longer degradation half-life values, ranging from 2 to over 15 years (64, 65). Some compounds may leach below the soil surface and become unavailable or, over time, compounds may become strongly adsorbed to the soil matrix, resulting in stabilization to photolytic degradation. Photooxidation is also assumed to occur on soil surfaces; however, very little is published on this subject due to the complexity of the system (37).

Romero et al. (66) investigated degradation of phenoxyacetic acids (i.e., mecoprop and dichlorprop) in soil samples of different humidity and organic matter content. Based on the conducted investigations, it has been concluded that the removal rate of dichlorprop (DCPP) and mecoprop (MCPP) depends on soil humidity; that is, the rate is much slower in dry soils. The photochemical decomposition of MCPP occurs much faster than in DCPP, which might be likely explained by different abilities of these compounds to be adsorbed by soil.

Biodegradation

Biological decomposition of persistent organic pollutants by microorganisms is one of the most important and effective ways to remove these compounds from the environment. Usually, the biodegradation of organic compounds is a multistep process, which is taking place in the presence of a number of microorganisms that often act synergistically. Biodegradation rate in real aquatic environment depends on characteristics of the aquatic system, presence of particulate matter, concentration of inorganic and organic nutrients, temperature, oxygen concentration, redox potential, and adaptation of the microbial population. In soil, the range and rate of biodegradation processes depend on several factors such as, soil temperature, soil moisture content, the composition and activity of bacterial flora, the properties and "age" of a pollutant, and the content of nutritive ingredients (19, 33).

Microorganisms, in comparison to other organisms, have a particular predisposition to adapt to novel environmental conditions and the ability to utilize compounds that are not the products of their own metabolism, as substrates needed for energy production and structure building. In general, microorganisms can be divided into autotrophic, i.e., these, which use carbon dioxide as a carbon source, and heterotrophic ones that obtain carbon from decomposition of organic matter and man-made organic substances. The processes in the organic compounds take place due to a direct contact with the microbial cellular enzymes.

One molecule of enzyme can catalyze decomposition of millions of organic molecules per minute (67). The reactions mediated by microorganisms are, to a large degree, similar to those occurring in higher organisms. Therefore, the aromatic compounds undergo epoxidation and hydroxylation, the aliphatic ones are oxidized and degraded through the β–oxidation pathway, and the nitro-organic derivatives are metabolized with the use of nitroreductases. Microorganisms can also mediate the processes that the higher organisms are not capable of, e.g., decomposition of aromatic ring or dehalogenation.

In general, there is a relatively large variety of microorganisms in the natural environment; a higher diversity has been observed in the microbial biocenoces associated with sediments than in groundwaters originating in formations of large pore size and inhabited by mobile microorganisms. Microorganisms residing in sediments are sedentary, permanently bound and living in pores of small diameter. The characteristic morphological types are gram-negative rods, among others, aerobic rods from the strain *Pseudomonas, Flavobacterium, Azotobacter*, and *Rhizobium*, nonobligatory anaerobic species *Aerobacter aerogenses*, and anaerobic species from the genus

Desulfovibrio. From among the gram-positive bacteria, typical for this environment is the genus *Arthrobacter*, as well as bacterial spores from the aerobic genus *Bacillus* and anaerobic genus *Clostridium* (6).

Biodegradation of Organic Compounds in Soil and Benthic Sediments

Soil is a mixture of organic, mineral, gaseous, and liquid components, inhabited by numerous microorganisms. Organic matter in soil consists of the remnants of decomposing plants and humic substances. The mineral components of soil are particles of weathering rocks, silt, and hydrated oxides of Al. Organic compounds are decomposed by microorganisms that live in soil; however, the rate of decomposition depends on the properties of an organic substance, microbial genotypes, pH, nutritive properties of soil, soil temperature, soil moisture content, and soil adsorption characteristics. Enzymes cause microbial decomposition of substances and, it generally occurs after an initial latency period during which the microorganisms adapt to novel substratum. In many cases, the reactions taking place in soil are similar to those occurring in sediments, although, usually only the surface layer of sediment contains oxygen. Most of the sediment constitutes the anaerobic compartment (62).

Polycyclic aromatic hydrocarbons get transferred into the environment as a result of the incomplete combustion of organic substances at high temperature (68). The ability of microorganisms to degrade polycyclic aromatic hydrocarbons depends on the number of aromatic rings in a given compound. Microorganisms can decompose compounds with 2 to 4 benzene rings and, at the same time, use the process as a carbon source. A larger number of rings, i.e., 5 to 6, give the compound higher resistance to microbial "attacks" (69, 70).

The research of degradation processes of selected PAHs by various strains of aerobic bacteria has been described by Yuan et al. (71). Experiments that were conducted for 5 different PAHs being decomposed in a mixture, separately, showed that phenanthrene, acenaphthene, and pyrene were completely degraded within 28 hours, 10 days, and 12 days, respectively; at the same time, the content of anthracene and fluorene practically did not change. After mixing all the compounds together, a decrease of phenanthrene, acenaphthene, and pyrene biodegradation was observed, while the rate of the process for anthracene and fluorene showed an increase. This has proved that the compounds of lower molecular weight degrade much faster as compared to the compounds characterized by high molecular weight. The same authors investigated the subject of phenanthrene biodegradation in riverine sediments (25). Results obtained indicate that the phenanthrene biodegradation rate varies depending on the values of pH and temperature, with the optimal rate reached for 30°C and pH = 7.0. The additional substances, such as sulphates and phosphates, did not show an effect on the degradation rate.

Based on scientific information, it has been concluded that biological treatment of soil in order to eliminate PAHs of lower molecular weights (e.g., naphthalene, phenanthrene, and pyrene) is very effective because many microorganisms capable to utilize such compounds as an energy and carbon source were identified and researched (Figure 7) (69, 70, 72–74).

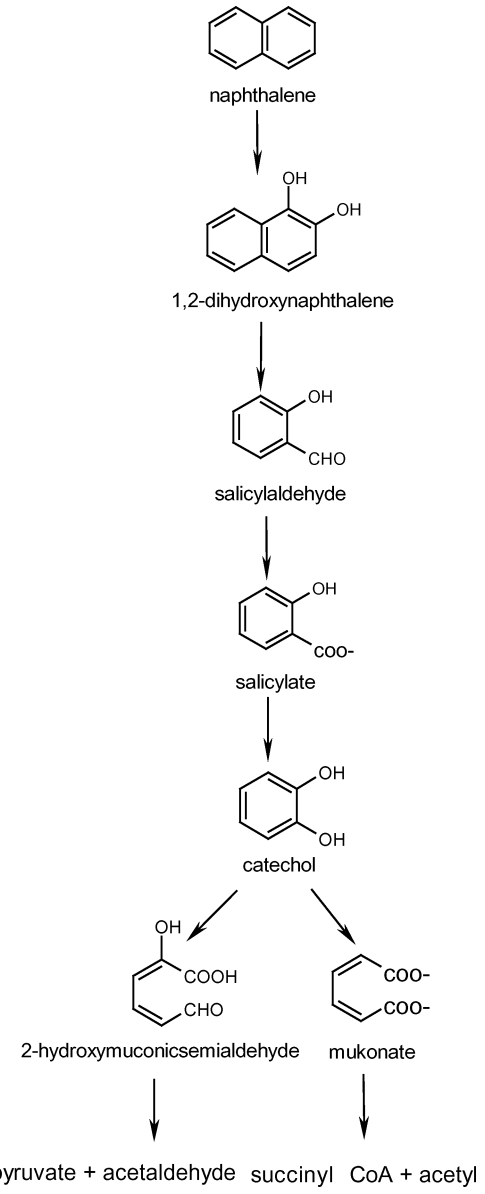

FIG. 7. Schematic pathway of naphthalene degradation by bacteria from the genus *Pseudomonas* under aerobic conditions (9).

Juhasz et al. (72) have investigated the biodegradation of benzo(a)pyrene, dibenzo(a,h)anthracene and coronene, all being compounds of high molecular weights, by using a strain of *Burkholderia cepacia* (VUN 10,001). The used bacterial strain degraded PAHs with 5 and 7 aromatic rings; however, the rate of the process was much lower as compared to that for pyrene. A noticeable 20–22% decrease of benzo(a)pyrene and dibenzo(a,h) anthracene content was observed after only 63 days

of incubation. In case of coronen, after 63 days, its concentration decreased by 75%.

Aerobic degradation of PAHs in sediments has been extensively documented by Cullen et al. (75) and Geiselbrecht et al. (76). Verification of anaerobic degradation of PAHs under nitrate-reducing conditions has been presented by Bregnard et al. (77) and Langenhoff et al. (78). Chang and coworkers (79) suggest that anaerobic microorganisms might have greater potential for organic-pollutant detoxification in the environment. Comparison of phenanthrene degradation under three reducing conditions was done and it has been the order of phenanthrene remaining for sediment sample are: nitrate-reducing conditions > sulfate-reducing conditions > methanogenic conditions. It has been also found that additional acenaphthene and phenenthrene was completely degraded in sediments within 56 days incubation, respectively, while pyrene, fluorene, and anthracene were degraded only 4.0, 28.0, and 48.7% within a 56-day incubation period. High to low degradation rates were phenanthrene → acenaphthene → pyrene → fluorene → anthracene. According to Walton and Anderson (80), PAH bioavailability and biodegradability depend primarily on the complexity of their chemical structures and corresponding physicochemical properties.

Biodegradation of polychlorinated biphenyls (PCBs) is a multistep process that involves aerobic and anaerobic bacteria. Anaerobic bacteria are capable to decompose compounds containing several chlorine atoms while anaerobic bacteria only degrade compounds with one or two chlorine atoms. The first step of the process is degradation of polychlorinated compounds during which bacteria do not use PCB as a carbon source, but rather as an electron acceptor. The derivatives with one or two chlorine atoms, originating in the process, undergo further decomposition through breaking of the aromatic ring in the presence of aerobic bacteria. The ensuing reactions lead to the production of inorganic chlorine, carbon dioxide and water, as stated by Müller and Lingens (81). Figure 8 shows a generalized pathway of microbial degradation of PCBs.

Immobilized cells of *Pseudomonas sp.2* are able to degrade di-, tri-, and even tetrachlorobiphenyls. According to Komancova (82) 52–99% of original PCBs was degraded after 3 weeks. For all tested congeners, chlorobenzoic acids were found as degradation products. The most common and the most investigated pathway of PCBs transformation is 2,3-dioxygenase attack starting by the oxidation of less chlorinated ring (82). White rot fungi (*Pleurotus ostreatus*) had biodegradation ability of low chlorinated PCBs. *P. ostreatus* strains decomposed PCBs selectively with the preference for congeners with chlorine atoms in ortho>meta>para positions. Degradation efficiency decreased with increasing number of chlorination (83).

The potential for the anaerobic degradation of three PCB congeners (2,3,5,6-CB; 2,3,4,5-CB, and 2,3,4,5,6-CB) in sediments was investigated by Chang et al. (84). According to the authors, intermediate 2,3,4,5-CB products were identified as 2,3,5-CB, 2,3,6-CB, and 2,5-CB. For 2,3,5,6-CB, intermediate products were identified as 2,3,6-CB and 2,5-CB. Dechlorination rates for PCB congeners were observed as, ordered from the fastest to the slowest: 2,3,4-CB > 2,3,4,5-CB > 2,3,4,5,6-CB > 2,2′,3,3′,4,′-CB > 2,2′,4,4′,6,6′-CB > 2,2′,3,4,4′,5,5′-CB > 2,2′,3,3′,4,4′,5,5′-CB. The rates decreased for mixtures of the eight congeners. Dechlorination rates for three primary congeners under different reducing conditions occurred in the following order (from the fastest to the slowest): methanogenic condition > sulphate-reducing condition > nitrate-reducing condition. Under methanogenic and sulphate-reducing conditions, dechlorination rates were enhanced by the addition of lactate, pyruvate or acetate, but decreased as a result of the addition of manganese oxide or ferric chloride. Under nitrate-reducing conditions, dechlorination rates were decreased by the addition of lactate, pyruvate, acetate, manganese oxide, or ferric chloride. The dechlorination of the three PCB congeners was affected by changes in pH, temperature, and the presence of an electron donor or acceptor.

Biodegradation of Organic Compounds in Ground Waters and Surface Waters

Phenoxyacetic acids are herbicides widely used in agriculture. These compounds are selective because grains are resistant to their herbicidal properties while leafy plants display sensitivity. Herbicides get into waters by means of diffuse run-offs from agricultural fields.

Harrison et al. (85) investigated degradation rates, under aerobic and anaerobic conditions, in 3 herbicides, namely, derivatives of phenoxyacetic acid, i.e., MCPA, present in groundwater. The experiments were conducted by using ISM technique (in situ microcosms) (86, 87) while the final determinations were performed by HPLC. Based on the experimental results it has been concluded that during aerobic degradation all herbicides underwent complete decomposition. Under anaerobic conditions, in the course of 100–200 days of the experiment, no changes in the content of selected phenoxyacetic acids were observed (85).

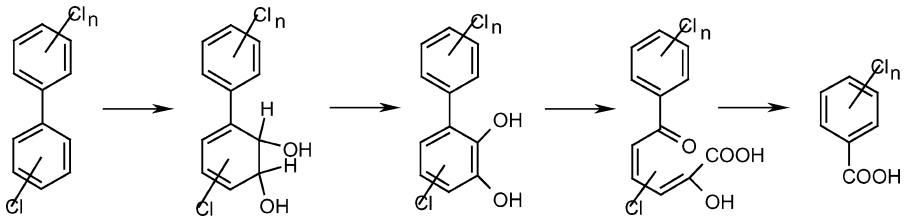

FIG. 8. Microbial degradation pathway of polychlorinated biphenyls (81).

The degradation of sulfonylurea herbicide rimsulfuron and its major metabolites in freshwater was investigated by Martins et al. (23). They investigated that in aqueous solutions, rimsulfuron is rapidly hydrolyzed into metabolite 1, which itself is transformed into the more stable metabolite 2. Metabolite 2 is more persistent ($t_{1/2}$ = 8.1–55 days, depending on initial concentration) and can present a potential danger to groundwater.

The phenomena connected to degradation of polycyclic aromatic hydrocarbons in water have also been discussed in many studies. Microbial decomposition of selected PAHs in samples of water collected from wells was investigated by Ogawa et al. (14). The compounds degraded almost completely within 3 days. The removal rates of particular PAHs have been ranked as follows: acenaphthylene > acenaphthene > 2-methylnaphthalene > 2-methylindene > 3-methylindene > lindane.

Garon et al. (88) investigated the degradation of fluorene by different fungal strains, isolated from soil, that have been known to effectively degrade PAHs. Water samples containing different fungal strains and contaminated with fluorene were incubated for 2 days, and later analysed by using HPLC. Twelve of the investigated strains degraded 60% or more of fluorene; three strains from the family *Cunniganella* achieved 96% degradation. In all cases, the main products of fluorene degradation were 9-fluorenol and 9-fluorenone. Both newly formed products were identified based on a comparison of retention time and absorption spectrum against the standardized reference materials. The pathway of fluorene biodegradation is presented in Figure 9.

Ravelet et al. (90) researched the degradation of pyrene by using white-red fungi. Forty-one fungal strains were isolated from the sediments contaminated with PAHs, and later identified and classified into several taxonomic groups, i.e., *Zygomycetes, Deuteromycetes, Dematiaceae*, and *Sphaeropsidate*. Pyrene-containing samples were incubated for 2 days, and afterwards, the content of the remaining compound was measured. For 10 of the isolated strains a large decrease of pyrene was observed while *Zygomycetes*, particularly *Mucor racemosus*, has been recognized as the group most effective at pyrene decomposition. A *Penicillium crustosum* strain was the only one not capable to degrade pyrene at all. Among the 10 pyrene-degrading fungi, nine have not been described previously in literature.

Gram-negative bacterium (strain MV1) has been used to degraded bisphenol A (BPA) in aquatic environment (91). Two primary metabolites of BPA degradation by the strain MV1 have been found out: 4-hydroxybenzoic acid and 4-hydroxyacetophenone in the major pathway, and 2,2-bis(4-hydroxyphenyl)-1-propanol and 2,3-bis(4-hydroxyphenyl)-1,2-propanediol in the minor. The rate of BPA degradation depends on bacterial counts of the samples.

The changes in the mutagenicity of fenitrothion during its biodegradation in solutions were investigated by Matsushita et al. (92). Fenitrothion is completely decomposed within 12 days. The mutagenicity increased during anaerobic biodegradation, which was due to amino-fenitrothion, a metabolite formed during anaerobic biodegradation of fenitrothion. In case of chlornitrofen mutagenic metabolites were formed during anaerobic biodegradation, too (93).

Toxicity of Degradation Products in Comparison with Toxicity of Primary Compounds

Before degradation will reach the endpoint, there are intermediate products being degradation products of primary compounds. At the moment it is of great importance to identify and quantify these products because frequently, the intermediate products of the degradation of organic compounds show much higher toxicity to microorganisms, animals, and humans than the parent compounds.

The degradation of some PAHs may serve as a superior example. Some of these compounds cause the formation of DNA and RNA adducts, and that, in turn, stimulates the growth of cancer cells and mutagenic changes that begin the alteration of the genetic material to be later inherited by the offspring. PAHs, which enter an organism, undergo oxidation processes mediated by hepatic enzymes, and the following metabolites are being produced: epoxides, diols, phenols, and quinones. Particularly hazardous are the epoxide derivatives. The changes to which PAHs are subjected can be depicted by a metabolic pathway of benzo(a)pyrene in a living organism at presented in Figure 10.

Benzo(e)pyrene, a product of microbial oxidation of benzo(a)pyrene, has strong carcinogenic properties (62). Based on the research of environmental effects resulting from its metabolic pathway, it has been proved that some plant and animal species are much more susceptible to the forming metabolites than to the parent compounds. As said by Coats (95) earthworms are 6 times more sensitive to *p*-nitrophenol and 14 times to 2,4-dichlorophenol than to parathion and 2,4-D, respectively. Examples of metabolic changes of 2,4-D and parathion are shown schematically in Figures 11 and 12.

Organophosphorus pesticides, when presented in natural waters, degrade into compounds that also have activity against pests. The few studies indicate that the degradation products may exhibit higher, lesser, or similar activity to the parent pesticide. For example, as stated by Pehkonen and Zhang (97) degradation of chlorpyrifos to 3,5,6-trichloro-2-pyridinol (via hydrolysis) results in a total loss of insecticidal activity, nevertheless the product is bioactive against several fungal pathogens.

In contrast to the above-mentioned examples, data from Wei et al. (98) indicate that for sulfonylureas, which are easily degraded into substituted sulfamine and heterocyclic compounds in environment, acute toxicity to the cladoceran *Daphnia magna*, a primary consumer in fresh water ecosystem, has been tested and showed that herbicides are more toxic to both *P. phosphoreum* and *C. pyrenoidosa* than their degradation products.

Also degradation product of Irgarol 1051 was found to be less toxic to the crustacean and the microalga than Irgarol 1051

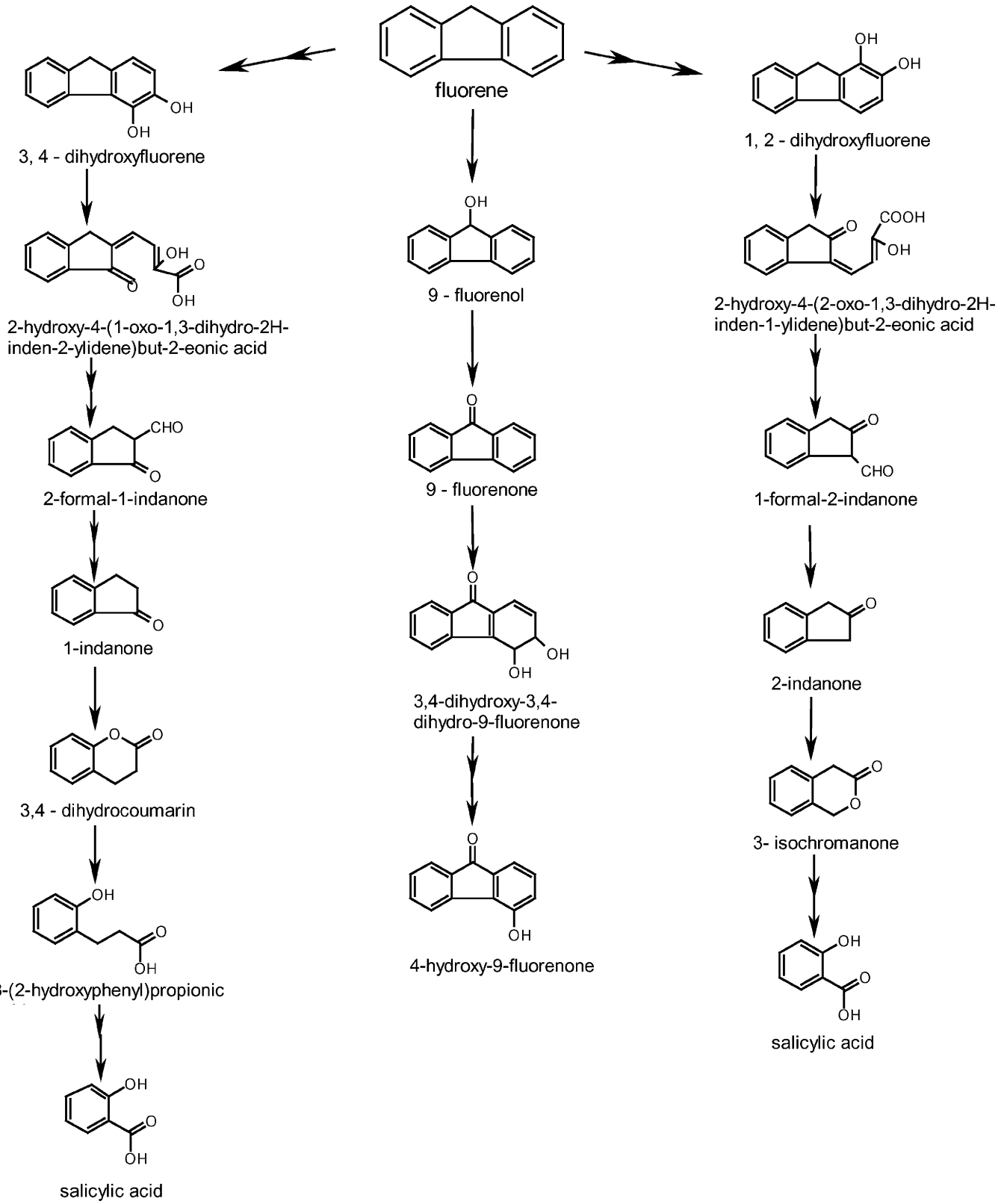

FIG. 9. Degradation pathway of fluorene (89).

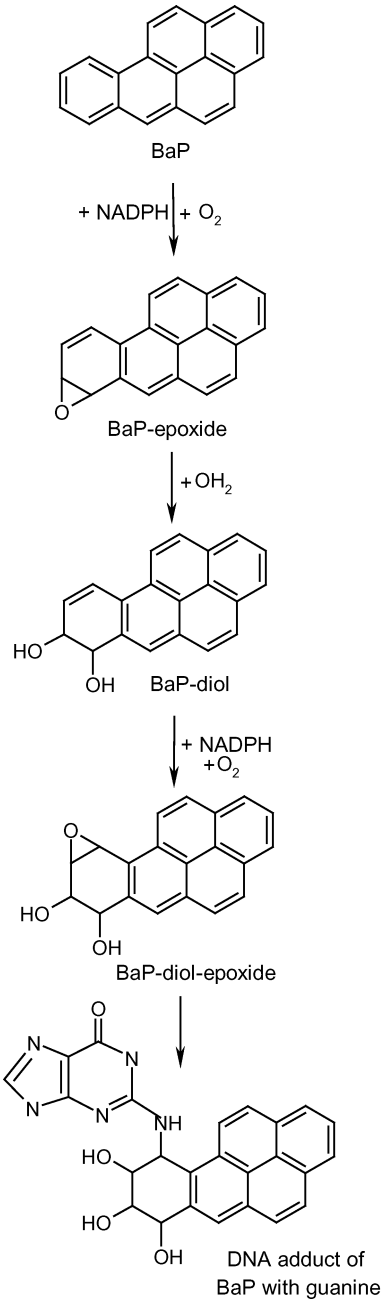

FIG. 10. Metabolic pathway of benzo(a)pyrene (BaP) in living organisms (94).

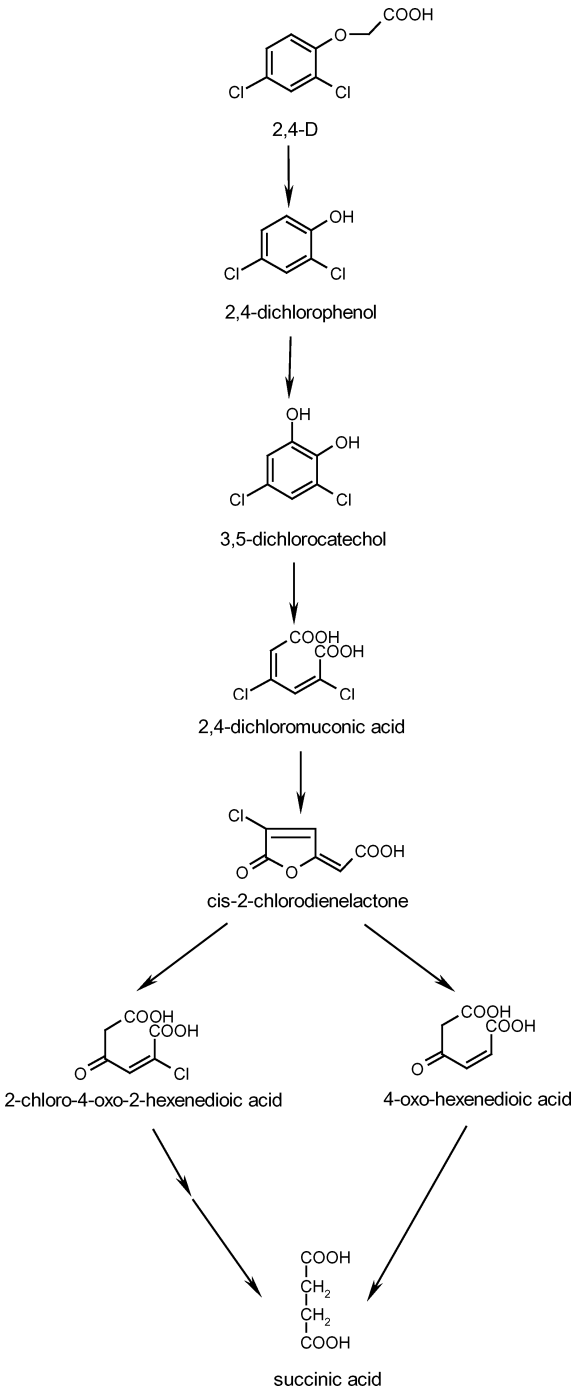

FIG. 11. A schematic pathway of biodegradation of 2,4-dichlorophenoxyacetid acid (2,4-D) (81).

itself but more toxic to the bacterium, as it has been published by Fernández-Alba et al. (99). According to them degradation products of diuron were less toxic to the microalga in comparison with the bacterium. For the mixtures of compounds (Irgarol 1051 and diuron), toxicities were additive in only 33% of the cases, and 21% of mixtures were less toxic than expected, based on the sum of concentrations of toxicants (antagonistic effect). Synergistic enhancements of toxicity were observed for the majority of mixtures, i.e., for 46% of them. The toxicity of compounds was measured for single compounds and for the mixtures of various complexities, using acute toxicity bioassays. As different toxicants act differently and not all life forms are equally susceptible, several bioassays were used to assess the toxicity. Ferrer and Barceló (100) identified degradation products of Irgarol 1051.

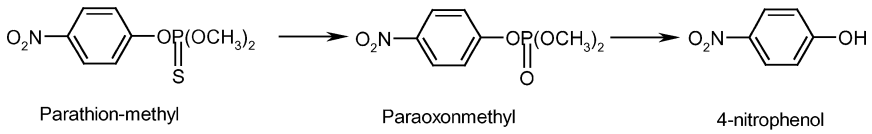

FIG. 12. One of the possible degradation pathways of parathion (96).

ANALYTICAL TOOLS USED IN STUDIES OF DEGRADATION PROCESSES

Studies on degradation processes are carried out with a focus on different aspects. Thus, the identification of products resulting from degradation processes, kinetics studies, the determination of mutagenicity and toxicity, as well as description of DNA adducts formed by POPs, are of the main interest.

Analytical measurements in this particular area play an important role because of:

- variety of organic compounds classified as environmental pollutant;
- variety of several stages processes involved in degradation and variety of intermediate products;
- complicated environmental sample matrices;
- the presence of compounds frequently at high level, which interfere with analytes; and
- very low level of the most organic pollutants in environmental samples.

Monitoring of pathways of organic pollutants and their degradation products and degradation processes in the environment is performed using a wide range of analytical techniques. Sample preparation methods used before quantitation step are of the highest importance.

Kinetics Studies. In order to determine the lifetimes of organic chemicals, under the assumption that degradation mechanism is being present kinetics studies are carried out. Various analytical techniques are applied to monitor these studies, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), ion exchange chromatography (IEC), total organic carbon (TOC) analysis, UV-visible spectrophotometry, spectrofluorimetry, radiometry, electron paramagnetic resonance (EPR), spectroscopy, and Fourier transform infrared (FT-IR) (8).

Toxicity. The identification of products resulting from biodegradation represents a difficult and time-consuming process due to the complex composition of biodegradation mixtures. Moreover, identification of particular product and its potency in a toxicological assay does not necessarily reflect the toxic or mutagenic effect of the compound as a part of biodegradation cocktail. Therefore, evaluation of the toxicity of fractions of degradation products in addition to the original compounds and its main degradation product is recommended. It is obvious this approach is not universal, however it may serve for prediction of a harmful effect caused by the application and subsequent degradation of particular compound in the environment. This study is based on toxicity assessment, which was evaluated as a decrease of intracellular ATP/ADP in human epithelial cells. This method is a simple technique for evaluation of possible risk of intoxication by xenobiotics (101).

Mutagenicity. The Ames test is a very well known test for determining if a chemical is a mutagen. It is stated that the use of the Ames test is based on the assumption that any substance that

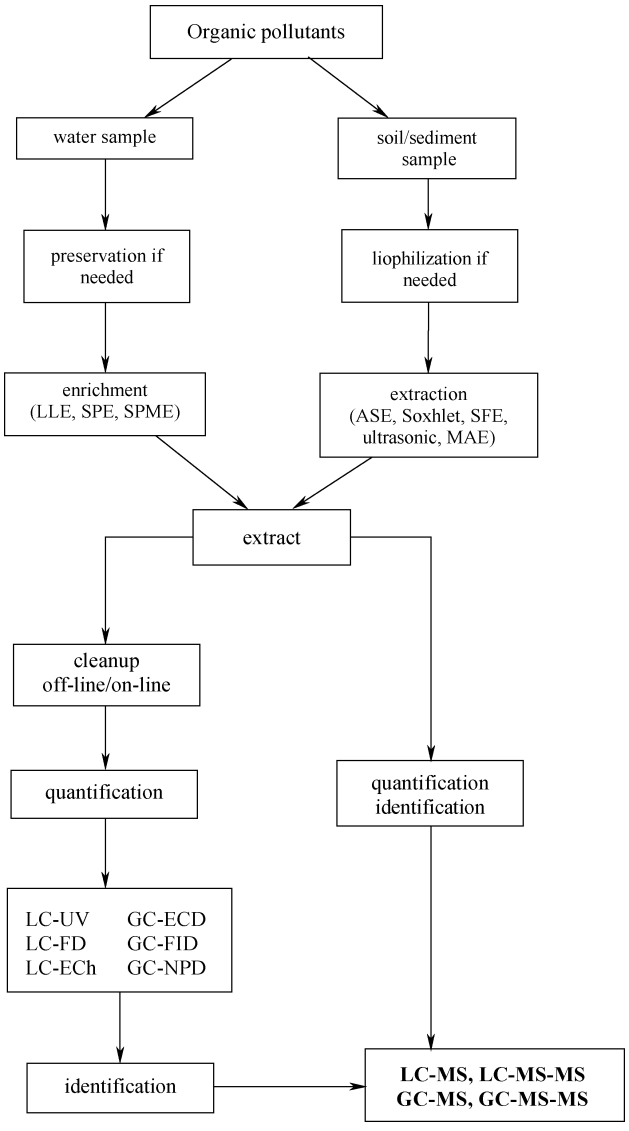

FIG. 13. Comparison between the existing method and the LC(GC)-MS method for the assay of organic pollutants in different media.

TABLE 1
Analytical procedures for determination of POPs and their degradation products in environmental samples

Analytical tools	Compounds	Sample matrix	Enrichment procedure	Reference
HPLC-UV/DAD	Phenoxyacetic acids mecoprop; MCPA; 2,4-D; 2,4,5-T	Ground waters and surface waters	SPE-Isolute C18 and LiChrolut EN cartridges	(110, 112, 122)
	Pesticides atrazine, simazine, carbendazim, Irgarol	Soil, water	Soxhlet extraction; SPE-Carbograph 4, LiChrolut EN cartridges	(109, 123, 124)
	PAH Pyrene, fluorene, anthracene	Water, soil, benthic sediments	Soxhlet extraction with ethyl acetate; SPE-Bond Elut 2OH, C18 cartridges; LLE with dichloromethane Extraction with ethyl acetate	(49, 73, 88, 125)
	Chlorophenols pentachlorophenol	Soil		(126)
HPLC-FL	PAH anthracene, phenanthrene, fluorene, pyrene	Water, soil	SPE Extraction with bidistilled ethyl acetate	(88, 127–129)
HPLC-MS	Phenoxyacetic acids MCPA; 2,4-D; chloroacetanilide	River water, surface waters, soil	SPE-C18, Carbograph 1 cartridges Extraction with KOH	(111, 117, 130, 131)
	Pesticides amidosulfuron, bentazone, propiconazole atrazine, simazine, propazine, anachlor diuron	Ground waters and surface waters	Direct injection; SPE C18, SDB-1 cartridges	(52, 111, 121) (124, 132–134)
		Soil	Soxhlet extraction; MAE	(135)
	Chlorophenols 4-chlorophenol, 2,4-dichlorophenol 2,4,6-trichlorophenol, pentachlorophenol			
GC-MS	PAH antracen, fenantren, naftalen, piren, fluoranten	Benthic sediments Water Coal tar	LLE with ethyl acetate; extraction with dichloromethane	(69, 115, 119, 136, 137)
	Dioxins PCDD, PCDF	Aqueous solutions Benthic sediments	LLE with dichloromethane or benzene	(44, 138, 139)
	Polychlorinated biphenyls 4,4′-dichlorobiphenyl, 3,3′,4,4′-tetrachloro biphenyl 2,2′,4,4′,5,5′-hexachlorobiphenyl	Soil Aqueous matrices	Extraction with ethyl acetate; SPME-(polydimethyl siloxane fibre)	(140, 141)

(Continued on next page)

TABLE 1
Analytical procedures for determination of POPs and their degradation products in environmental samples *(Continued)*

Analytical tools	Compounds	Sample matrix	Enrichment procedure	Reference
	Chlorophenols pentachlorophenol 4-chlorophenol	Water	SPEC18 cartridges extraction with ethyl acetate	(142) (143)
MEKC	Pesticides atrazine, simazine, propazine	Mineral water, tap water	PS-DVB SPE disks SPE-C18 cartridge	(144, 145)
ELISA Method	Pesticides triazines alachlor, atrazine, simazine, metolachlor, bromacyl chloroacetanilide	Rain ground waters and surface waters	LLE with dichloromethane SPE-C18 cartridge	(105–107, 146, 147)
	PAH benzo(a)pyrene	Blood	DNA isolation with extraction with phenol, chloroform: isoamyl alcohol and diethyl ether	(104, 108)

is mutagenic for the bacteria used in this test may also turn out to be a carcinogen, i.e., it can cause cancer. Although, in fact, some substances that cause cancer in laboratory animals (e.g., dioxin) (92, 93) do not give a positive Ames test result (and viceversa). However, the ease and low cost of the test make it invaluable for screening substances present in our environment for possible carcinogenicity. The bacterium used in the test is a strain of *Salmonella typhimurium* that caries a defective (mutant) gene, making it unable to synthesise the amino acid histidine from the ingredients in its culture medium (102, 103).

ELISA Technique. The direct covalent binding of a carcinogenic agent to DNA to produce carcinogenic DNA adducts is an essential step in the development of cancer. DNA adducts formed by POPs can be detected with the use of Enzyme-Linked Immunosorbent Assay (ELISA)(104). It is a fast, reliable, cost-effective technique that can be conducted both in the laboratory and in the field (105, 106). The ELISA technique has been proved to be a reliable tool for screening some pesticides (e.g., atrazine) in groundwater samples, but not for other triazine herbicides and their degradation products because of the relatively low assay specificity (107). In most studies described so far ELISA is also used for the measurement of PAH-DNA adducts in human cells (108).

Identification of Degradation Products. The chromatographic techniques, mainly GC and HPLC directly coupled with mass spectrometer (MS), are the most frequently used methods of identification and determination of persistent organic pollutants and their degradation products present in the environment (8, 52, 72, 109–117).

In literature, there are detailed data published by Pozo et al. (117) showing the determination of the contents of 4-chloro-2-methylphenoxyacetic acid (MCPA) and its main derivative, 4-chloro-2-methylphenol, in water and soil, performed by LC-MS technique. High selectivity and sensitivity (detection limit for MCPA equals 40 ng/l) together with short time required for sample analysis, i.e., 14 minutes, proves that LC-MS technique is a fast and reliable method for determining such pollutants.

The products of herbicide degradation, mainly 2,4-dichlorophenoxyacetic acid (2,4-D) have been investigated in caterpillars from the American species *Eupackardia calleta*. According to Deml and Dettner (118) after adding 2,4-D to the feeding medium, the compound's fate was monitored in the bodies of animals during the entire developmental cycle of the caterpillar. The presence of 2,4-D and its derivatives was noted in adult animals and their offspring. The analysis by means of GC-MS allowed to establish a hypothetical degradation pathway of 2,4-D in *E. calleta*.

Gas chromatography with mass selective detection (GC-MS), working either with SCAN or in SIM mode, can be a reliable tool for the identification of metabolic products of PAHs. As reported by Šepič and Leskovšek (119), GC-MS is used as an analytical tool for identifying the biodegradation products of fluoranthene (namely 9-fluorenone-1-carboxylic acid, 9-fluorenone, 9-hydroxy-1-fluorene-carboxylic acid, 2-carboxybenzaldehyde, benzoic acid and phenylacetic acid), a typical model of four ring polycyclic aromatic hydrocarbon, degraded by pure bacterial strain *Pasteurella sp.* IFA.

Vialaton and coworkers (52) studied the photolysis of propiconazole in pure water, in water containing humic substances and in natural water, and identification of the main photodegradation products was based on 1H NMR and HPLC-MS analyses.

Liquid Chromatography with Amperometric Detection has been an effective analytical tool for determination of nitroaromatic photodecomposition products in samples with complex

matrices due to its high reduction selectivity. This approach can be very advantageous in analyses of samples that contain not only the nitroaromatic compounds but also high concentrations of other substances (e.g., phenols, chlorophenols, or amines) (120).

An important aspect to consider when performing residue analysis at the low concentrations, relevant to soil and environmental waters is to assure a high degree of confidence in the identification of the compounds in order to avoid false positives. The MS fragmentation pattern is a powerful tool for obtaining such confidence in compound identification. However, by using tandem mass spectrometric detection, a more selective fragmentation of the initially formed deprotonated molecular ion is achieved. While LC-MS-MS is the method of choice in quantitative bioanalysis, it is still used to only a very limited extent in environmental analysis. Nevertheless, MS-MS for environmental analysis is gradually becoming more important, mainly in analytical strategies directed at rapid analysis (117). However, LC-MS-MS instruments are much more expensive in comparison to current conventional LC detectors. Hence, the replacement of existing methodology by LC-MS or LC-MS-MS procedures will depend on cost reduction in time of sample pretreatment, chromatographic run time and method development time.

Figure 13. Comparison between the existing method and the LC(GC)-MS method for the assay of organic pollutants in different media.

Another aspect to be considered is the effect of matrix on MS detection. For example, in the case of trace analysis of pollutants in environmental water samples, signal suppression caused by the presence of humic acids has been observed (121).

The most popular analytical procedures of determination of POPs and their degradation products are collected in the Table 1.

CONCLUSIONS

Degradation processes are natural and globally occurring due to decomposition of organic substances present in the environment. Degradation taking place in the collected environmental samples before the stage of final sample determination may, in a significant way, make it difficult or may bias the interpretation of the obtained analytical information. Thus, all processes possibly leading to sample degradation must not only be well known but also stopped.

A very important aspect of organic compound degradation in the environment is the possibility of applying these processes in natural and man-designed methods of pollution removal (remediation).

In both cases, it is necessary to know the processes in full detail, so that their effectiveness can be controlled, and their influence on analytical results can be reduced or eliminated.

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