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Review

The Importance of Degradation in the Fate of Selected Organic Compounds in the Environment. Part I. General Considerations

A. Kot-Wasik, D. Dąbrowska, J. Namieśnik*

Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology,
11/12 Narutowicz Str., 80-952 Gdańsk, Poland

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Abstract

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, surface and ground waters), they can undergo slow changes resulting from different chemical, physical, biological or photochemical processes. 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 are discussed.

Keywords: organic pollutants, degradation, products of degradation, determination, kinetics.

Introduction

Persistent Organic Pollutants (POPs), mainly emitted to the environment from anthropogenic sources, are in 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, surface and ground waters), 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 more or less harmful for the environment.

*Corresponding author; e-mail: chemanal@pg.gda.pl

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 in order to clean up 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 the 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

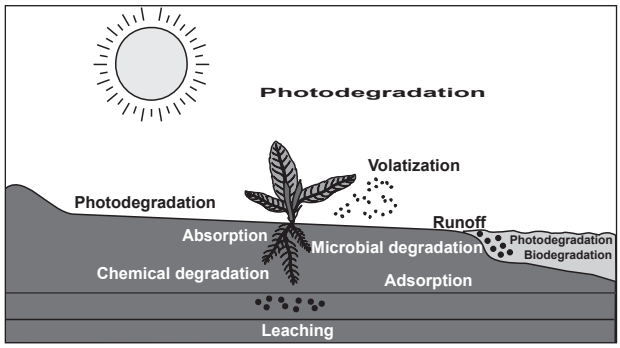

Fig. 1. Main processes to which persistent organic compounds are subjected in the environment.

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 which will lead to complete decomposition to carbon dioxide, water and inorganic salts [3]. The presentation of degradation processes going on in the environment is shown in Fig. 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 a 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 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 non-hazardous, 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 non-economical, 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 most economical technology available.

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 bacterial strains 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 a hundred times; however, the acceleration of bioremediation due to the 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

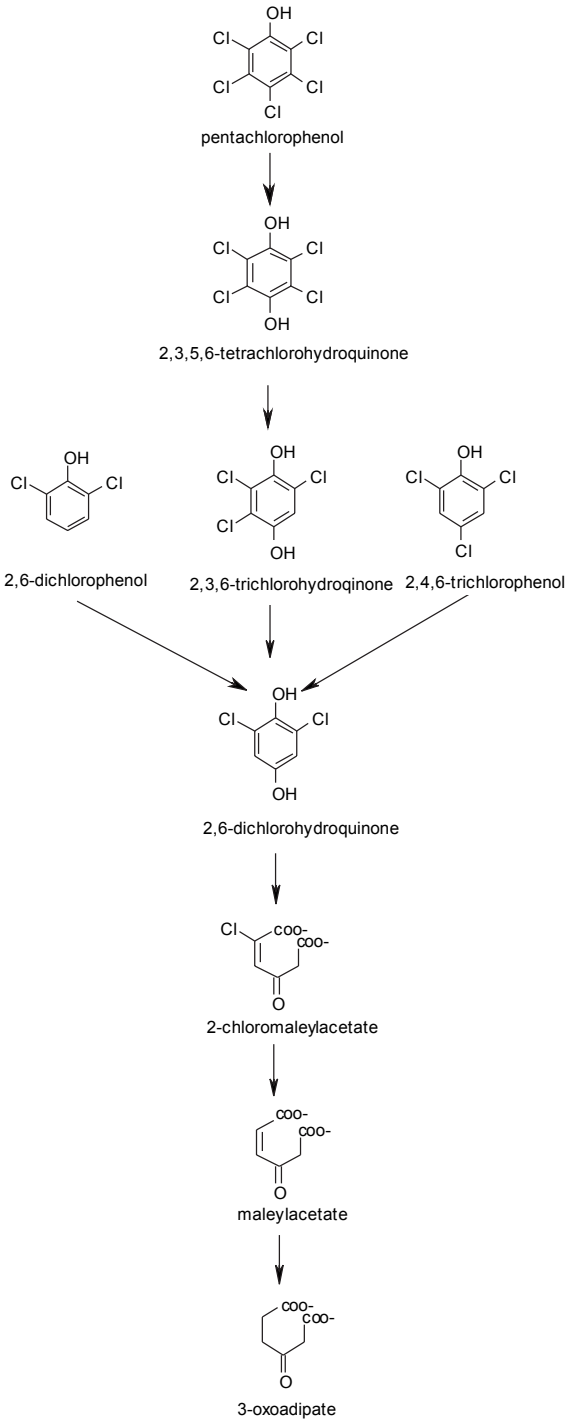

Fig. 2. Biodegradation pathway of PCP by *Flavobacterium* [9].

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 Fig. 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 high importance concerning the 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 a preventive action can be taken in order to fulfill 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 analysis (e.g. 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, particular attention has to be paid to these processes.

Undesirable changes in 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.

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Although choosing a proper method of sample preservation can minimize undesirable changes in sample composition, 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 which deal with the methods for sample preservation presented currently [10-18].

The Kinetics Degradation Processes

Even though POPs exhibit high persistency (here meaning the ability to stay unchanged in the environment for long time) it is well known that after some time, they start to degrade. Based on the literature assessment of degradation processes, degradation reactions are typically described by the first-order equation [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 with 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 followed also a 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 co-workers [29] studied the aqueous photodegradation of dichlofluanid. It was found that the photodegradation proceed *via* first-order reaction. As indicated by Penuela and Barceló [30] photodegradation of one of organochlorine pesticides, such as chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), is an effective method for removing 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, is used to compare the persistence of different chemicals with each other or with that of 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 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 values in subsoil and in ground water 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 build-up of environmental concentrations. This is illustrated in Fig. 3, which shows how environmental concentrations change for chemicals with different half-lives. The maximum environmental concentration reached for each substance depends on its half-life. Substances with short half-lives (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 towards background levels. On the other hand, for substances with long half-lives, the environmental concentration keeps on increasing. Even when emissions stop, the concentrations fall very slowly.

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

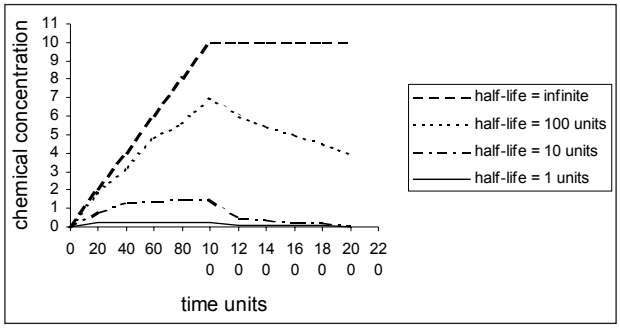

Fig. 3. Environmental concentrations of chemicals with various half-lives [31].

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 in order 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.

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, which begin the alteration of the genetic material to be inherited by the offspring later. 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 it is presented in Fig. 4.

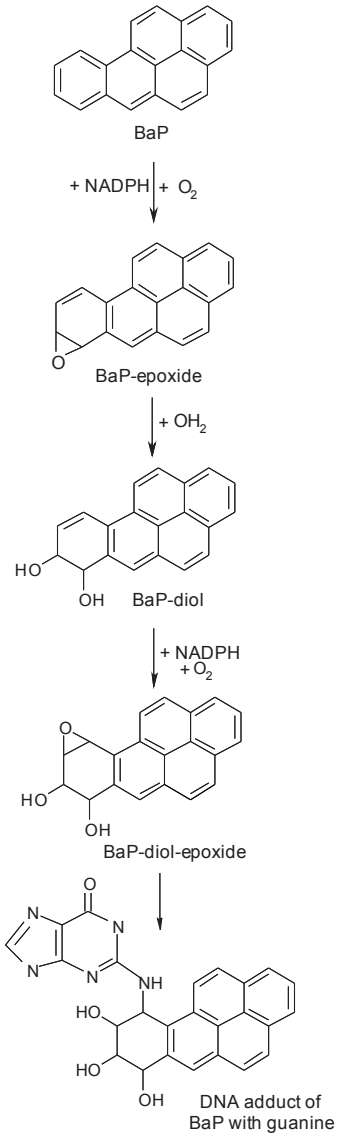

Fig. 4. Metabolic pathway of benzo(a)pyrene (BaP) in living organisms [32].

Benzo(*e*)pyrene, a product of microbial oxidation of benzo(*a*)pyrene, has strong carcinogenic properties [33]. 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 [34], earthworms are six 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 Figs. 5 and 6.

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 [37] 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.[38] indicate that for sulfonylureas, which are easily degraded into substituted sulfamine and heterocyclic compounds in the environment, acute toxicity to the cladoceran *Daphnia magna*, a primary consumer in freshwater ecosystems, has been tested and shown that herbicides are more toxic to both *P. phosphoreum* and *C. pyrenoidosa* than their degradation products.

Also, degradation of Irgarol 1051 was found to be less toxic to the crustacean and the microalga than Irgarol 1051 itself, but more toxic to the bacterium, as has been published by Fernández-Alba et al.[39]. According to

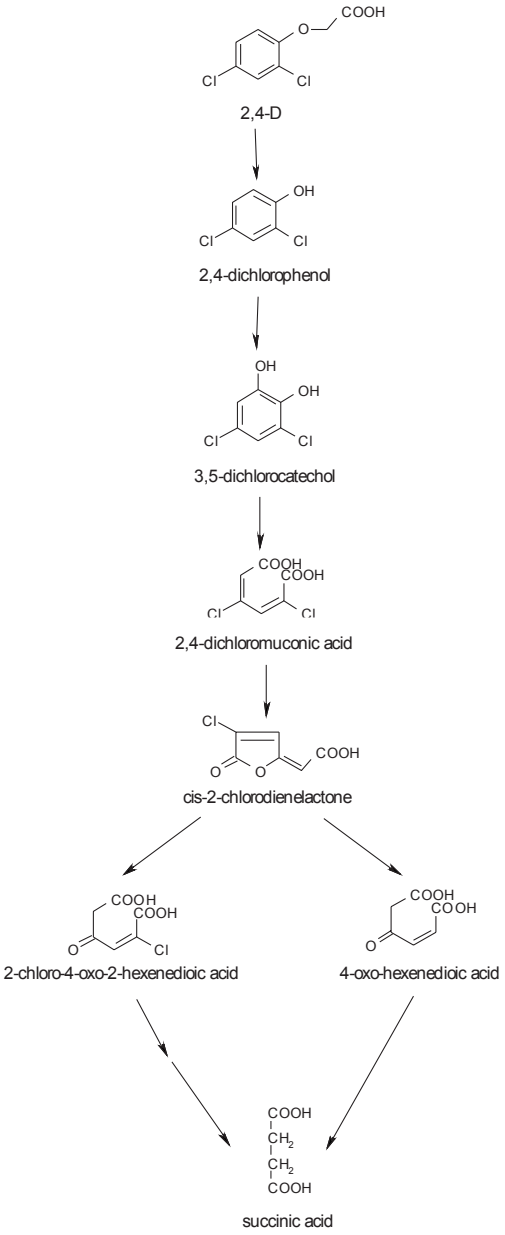

Fig. 5. A schematic pathway of biodegradation of 2,4- dichlorophenoxyacetid acid (2,4-D) [35].

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 [40] identified degradation products of Irgarol 1051.

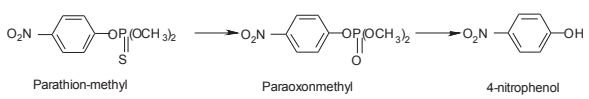

Fig. 6. One of the possible degradation pathway of parathion [36].

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 main interest.

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 Furier transform infrared (FT-IR) [41].

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 the 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 compounds in the environment. In such a way, 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 [42].

Mutagenicity

The Ames test is a very well known test for determining whether or not a chemical is a mutagen. It is stated that the use of the Ames test is based on the assumption that any substance that is mutagenic for the bacteria used in this test, may also turn out to be a carcinogen. Although, in fact,

some substances that cause cancer in laboratory animals (e.g. dioxin) [43, 44] do not give a positive Ames test result (and vice-versa). 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 [45-46].

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)[47]. It is a fast, reliable, cost-effective technique that can be conducted both in the laboratory and in the field [48,49]. 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 [50]. In most studies described so far ELISA is also used for the measurement of PAH-DNA adducts in human cells [51].

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 [52-63].

In literature, there are detailed data published by Pozo et al.[63] 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 the 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 [64] 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 [65], 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 co-workers [60] 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 ^{1}H 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 concentra-

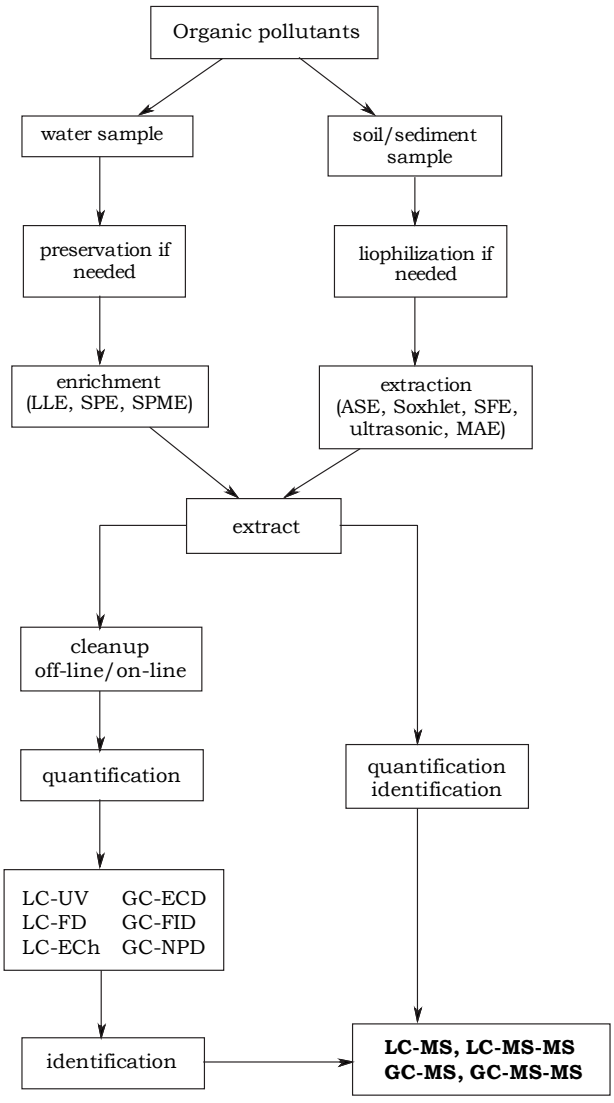

Fig. 7. Comparison between the existing method and the LC(GC)-MS method for the assay of organic pollutants in different media.

tions of other substances (eg phenols, chlorophenols or amines) [66].

An important aspect to consider when performing residue analysis at 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 [63]. 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.

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 [67].

Conclusions

Degradation processes are natural and globally occurring means of 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. Because of this, 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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