

1. Introduction

Several substances with high polluting potential are present in the environment and affect soil, sediments, water, air, microbial organisms, plants, animals, and humans. They may be distributed in one or all environmental compartments. A list of the most common and widespread pollutants is shown in Table 1.1. Polluting substances are very often present not only as mixtures of different organic compounds but also of organic and inorganic ones. The origins and sources of pollution are different: industrial activities such as mining and metal processing, petrochemical and industrial complexes, industry effluents, chemical weapons production, pulp and paper industries, dye industries and industrial manufacturing; and anthropogenic activities such as traffic, agricultural practices, and others. Pollutants may affect the health of humans, animals and environments for several causes. They may inhibit respiration. They may provoke a reduced reproduction of fish-eating birds as well as contribute to the birth of premature babies or children with genetic defects such as downs syndrome, anencephaly, and spina bifida. They may destruct reproduction in humans and animals, may have carcinogen and teratogenic effects on humans, and may give rise to arsenicosis and related damages.

Table 1.1 Organic, inorganic and air-pollutants possibly encountered in the environment

Organic	Inorganic	Air-Pollutants
Pesticides	Arsenic	Particulates
N and P derivatives	Cadmium	Greenhouse
PAHs	Chromium	gases
BTEX	Copper	Smog
PCPs	Lead	forming
NAPL	Cyanide	compounds
Plastics, Biopolymers		
Dyes		
Phenols,		
Chlorophenols		
Choroanilines		
Bleach plant effluents		
Nitrocompounds		

In 2007, the Blacksmith Institute of New York launched the second annual review of the most 2007 World's Worst Polluted Places - The Dirty Thirty Summary Matrix - which also included the top ten polluted sites. Almost all regions of the world have different and widespread types of pollutants and pollutant sources, i.e. Africa, China, Eastern Europe, Central and South Asia, Latin America and others (Blacksmith Institute 2007). Most of these areas are located in poor countries where pollution continues to be a major cause of death, illness and long-term damage. More than 200,000 polluted sites have been identified in developed countries such as Germany. In the Netherlands, 115,000 polluted sites have been estimated, though most of them have not been identified yet. These numbers give an idea of the enormous magnitude of pollution in the environment. And it could be said that a large part or even the entire earth is polluted.

As the increase of contaminated sites poses a major environmental and human health problem, it appears mandatory to decontaminate the environment and to implement efficient decontamination strategies. The main goals of decontamination should be recovery of soil health and fertility, detoxification of ground-water, reutilization of wastewater (mainly in countries with severe water deficiency), removal of negative effects on human and animal health, and production of healthy air. Therefore, a growing interest is being devoted to the search of effective remediation technologies for partial or total recovery of polluted sites. The nature of the contaminant sources and the co-occurrence of organic and inorganic compounds often make their remediation problematic.

Several methodologies have been applied for the remediation of polluted systems and many of them, when implemented in the target sites, have led to successful results. Two basic strategies have been utilized: engineering and biological ones (Bollag and Bollag 1995). Engineering strategies are basically founded on physical and chemical methods, whereas biological strategies require the involvement of biological agents (Gianfreda and Rao 2008). Composting, land farming, bioreactors, bioremediation, and phytoremediation are the main biological methods applicable to soil and

groundwater. Land farming is not suitable for the latter. Regardless the adopted method, the decontamination of polluted sites may be carried out by *in-situ* (if soils and water are treated directly on site) or *ex-situ* (if they are excavated, transported to another site and, then treated) treatments. Bioremediation and phytoremediation appear now as appealing technologies being based on the use of microorganisms, plants, and their enzymatic set.

Halogenated organic compounds are widely used as pharmaceuticals, herbicides, fungicides, insecticides, flame retardants, intermediates in organic synthesis, solvents etc. Additionally, many halogenated compounds are produced as by-products during chemical synthesis, such as dioxins, polychlorinated biphenyls and pentachlorophenol. Since many of these halo compounds show toxic or even carcinogenic effects and many of them are often difficult to degrade, many investigations were conducted to analyse the fate of these compounds in the environment. Studies on the degradation of different halogenated aliphatic and aromatic compounds led to the detection and elucidation of a variety of dehalogenases and dehalogenation mechanisms (Janssen et al., 1994; Fetzner 1998).

Halogenated aliphatics are detected almost anywhere. They are found in surface water [typical concentrations of trichloromethane, 1,1,1-trichloroethane, TCE, and PCE range from 0.1 µg /L to 6 µg /L (Bauer 1981; Correia et al., 1977), in groundwater (Canter and Sabatini 1994; Mayer et al., 1997), in food (Bauer 1981), and in the atmosphere. In urban air, the observed concentrations of dichloromethane were $0.03 \pm 0.1 \mu\text{L} / \text{m}^3$; 1,1,1-trichloroethane, TCE and PCE were found in concentrations of $0.1 \pm 0.6 \mu\text{L} / \text{m}^3$ (Rossberg et al., 1986). Contaminants found in public groundwater supplies (U.S. Superfund cases) were mainly volatile organic compounds such as TCE, PCE, 1,2-dichloroethene (DCE), vinyl chloride, and benzene. TCE and PCE were the most commonly found contaminants in water supply wells, detected in maximum concentrations of several to several hundred micrograms per liter (Canter and Sabatini 1994) Owing to the environmental impact of the pesticides and following the general parameters of toxicity, persistence and impact, several priority lists called “red” and/ or “black” lists

have been published (Gabaldon et. al., 1999). Among the various classes of pesticides that have been used in agriculture, organochlorine pesticides form a major group. These are pesticides which contain one or more chlorine atoms in the aromatic ring. As the number of chlorine atoms increase, the persistence and toxicity of the compound also increase. It also depends on the position of the chlorine atoms (Hardman 1991). 1, 1, 1- trichloro- 2,2 bis(4-chlorophenyl)ethane (DDT) is the best known of a number of organochlorine pesticides with 5 chlorine atoms in its molecule. It was used extensively during World War II by Allied troops in Europe and the Pacific as well as certain civilian populations to control insect typhus and malaria vectors. But DDT was less effective in tropical regions due to the continuous life cycle of mosquitoes and poor infrastructure. With continuous usage of DDT in malaria eradication programmes, the problem of residue accumulation started. In addition, mosquitoes also developed resistance to DDT. Spraying of DDT was curtailed due to the concerns over safety and environmental effects, as well as problems in administrative, managerial and financial implementation (Wikipedia 2006). Rachel Carson's book "Silent Spring" (1962) brought to light the ill effects of DDT on the flora and fauna. The book argued that DDT was poisoning both wildlife and environment and was also endangering human health. DDT is reported to be carcinogenic, mutagenic and teratogenic with estrogenic and androgenic effects (Aislabie et al., 1997). The pesticide is also persistent and recalcitrant leading to its bioaccumulation in the food chain. DDT and its metabolites enter the food chain leading to their biomagnification. The presence of the residues in food commodities is a major concern in both domestic and export business. The maximum residue levels, established by each country, govern the levels of these pesticide residues in all commodities. Hence detecting these compounds in the environment is of prime importance.

Though DDT is still considered as the wonder drug for preventing malaria and other vector borne diseases, its persistence in the nature and bioaccumulation resulting in the biomagnification ultimately is considered a health hazard. Upon introduction of DDT, it was appreciated all over the world but gradually, its ill effects came to light and restrictions on its usage started.

The Environmental Protection Agency (EPA) and other related organisations were seeking a complete ban on its use world wide. The ban on the usage of DDT was enforced in many countries. But, World Health Organisation (WHO) reintroduced the application of DDT in countries which were unable to combat malaria. As there is no alternative to DDT, its use in public health program continues (Dash et al., 2007). As the usage of DDT continues, the environment will be continuously fed by the residues of DDT. Hence, strategies for its detection and faster degradation need to be developed that are easily available at an affordable cost to all. The use of enzymatic proteins may represent a good alternative for overcoming most disadvantages related to the use of microorganisms (Nannipieri and Bollag 1991; Karam and Nicell 1997; Nicell 2001; Gianfreda and Bollag 2002; Gianfreda and Rao 2004). Enzymes have several beneficial characteristics. They are the main effectors of all the transformations occurring in the biota. They are catalysts with either narrow (chemo-, region- and stereo-selectivity) or broad specificity and, therefore, they can be applied to a large range of different compounds in mixture, as well. They may produce extensive transformations of structural and toxicological properties of contaminants, and even their complete conversion into innocuous inorganic end products. They may perform processes for which no efficient chemical transformations have been devised. Moreover, enzymes may present advantages over traditional technologies, and also over microbial remediation. They can be used under extreme conditions limiting microbial activity. They are effective at low pollutant concentrations and are active in the presence of microbial predators or antagonists. They act against a given substrate (microorganisms may prefer more easily degradable compounds than the pollutant), and are more mobile than microorganisms because of their smaller size (Gianfreda and Bollag 2002). All these characteristics render enzymes eco-friendly catalysts as well as enzymatic techniques are environmentally friendly processes. These latter may have the capability of remediation of many compounds that are unfriendly to the environment by the present ecological standards of our societies. As claimed by Alcade et al., (2006), biocatalysis by enzymes (very

often known as white biotechnology) “fully participates in the “green chemistry” concept introduced in the 90s by Sheldon and van Rantwijk (2004), and its effect on sustainability is now established beyond question”. Enzymes may act intracellularly or extracellularly, free form or immobilized (Gianfreda and Rao 2004).

The most representative enzymatic classes in the remediation of polluted environments are: hydrolases, dehalogenases, transferases and oxidoreductases. Their main producers are bacteria, fungi, mainly white-rot fungi, plants and microbe-plant associations. For many of these enzymes the transformation of different xenobiotic substances has been tested mainly under laboratory conditions. Reagents and activity assay conditions are available for many of these enzymes to allow their easy detection and application (Whiteley and Lee 2006).

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