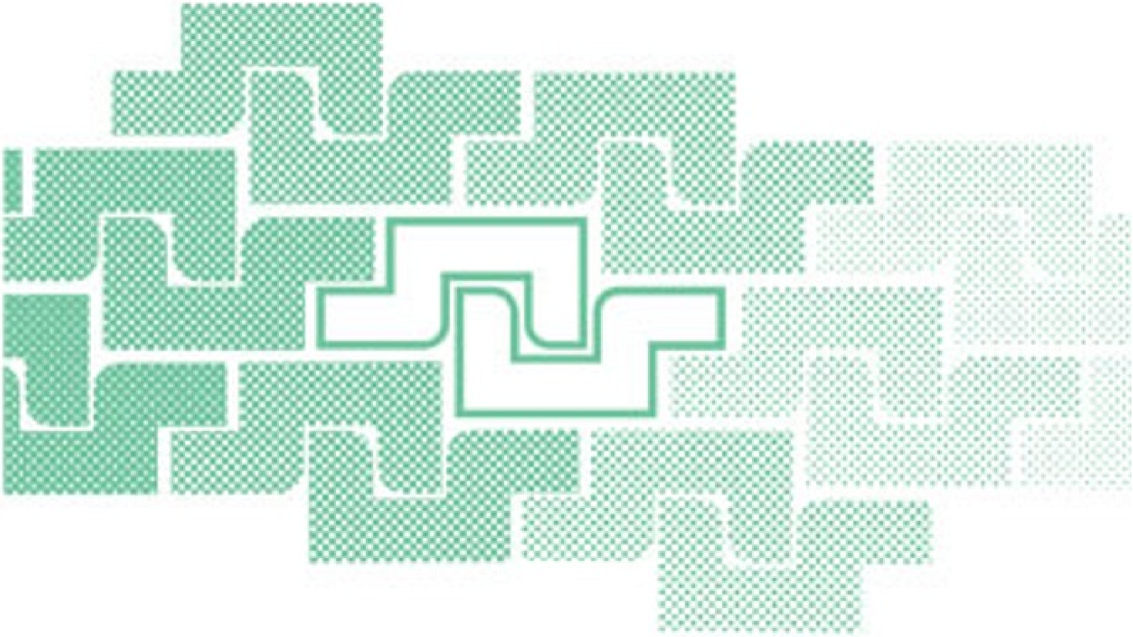

Soil and Water Pollution Monitoring, Protection and Remediation

Edited by

Irena Twardowska, Herbert E. Allen
and Max H. Häggblom

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PREFACE

> In every respect, human development and human security are closely linked to the productivity of ecosystems. Our future rests squarely on their continued viability.
>
> UNDP, UNEP, World Bank, World Resources Institute: *World Resources 2000-2001.People and Ecosystems. The Fraying Web of Life.*

1. OBJECTIVE OF THE BOOK

Soil, surface waters/sediments and shallow unprotected groundwater aquifers are interrelated compartments of the environment that are particularly easy to compromise, sensitive to short- and long-term pollution and directly affect sustainability of ecosystems and human health. Routine human activity such as application of fertilizers and pesticides in agriculture and forestry, or wet and dry deposition of atmospheric pollutants emitted from industrial plants, waste disposal and other practices adversely affect soil and water quality that already increasingly suffers from mismanagement in many areas. The predominant sources of pollution result in non-point contamination that is particularly difficult to reduce and control. Wars, accidents and natural emergency cases such as catastrophic floods that occur partly due to anthropogenically disturbed global water balance also add to overall increase of diverse contaminant loads in soil and water. Beneficial properties of some bulk waste materials such as biosolids (sewage sludge), biowaste (e.g. municipal waste composts) or fly ash from coal combustion encourage applying these waste to land as a source of nutrients and organic matter, or as a soil amendment. All these human activities result in soil being a sink for all possible chemicals, also new ones, that belong to many groups of xenobiotics - organic compounds such as halogenated organics (AOX), linear alkylbenzene sulfonates (LAS), Di(2-ethylhexyl)phtalate (DEHP), nonylphenol and ethoxylates (NPE), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-dioxins and furans (PCDD/F), organotins (MBT, DBT, TBT), and various pharmaceuticals. Heavy metals and metalloids, and some organic compounds, e.g. polycyclic aromatic hydrocarbons (PAH) that occur in nature but in much lower concentrations, are also of particular concern due to their persistence and toxicity. There is growing evidence of mutagenicity, genotoxicity and endocrine disruption impact of many chemicals on soil and aquatic organisms – primary receptors of soil and water contaminants - and their declining biodiversity. Other organisms – participants of the food web, including humans – have been also endangered.

During the history of human development and industrial revolution, mankind for a longer time ignored warning signs that its activity might damage the capacity of ecosystems to sustain us. Now, at the beginning of the third Millennium we are much more aware of our utter dependence on the productivity of ecosystems that is largely determined by the quality of their essential compartments: soil and water, and of the costs of their degrading and restoration. We also know much more about the processes that govern the fate and migration of contaminants in the environment. Nevertheless, we still know surprisingly little to be able to answer the basic question set up in the special millennial edition of the World Resources Report, *World Resources 2000-2001* (UNDP-UNEP-World Bank-World Resources Institute, 2000): "How best can we manage ecosystems so that they remain healthy and productive in the face of increasing human demands?" And how best can we manage soil and water resources to keep them healthy and productive?

We inherited a huge number of contaminated sites that have to be efficiently and cost-effectively restored, of different size and origin. We still continue to make mistakes in view of current benefits, and ignoring the precautionary principle and our limited knowledge. The development of this knowledge to enable correct prognosis of environmental behavior, bioavailability and impact of chemicals on living organisms is a priority task for better environmental management.

The data on long-term environmental behavior of many xenobiotics and efficient methods of their control are still scarce. Also data on heavy metals of anthropogenic origin are insufficient, in particular with respect to metals mobility, bioavailability and its alteration in time. New concepts and approaches in science that merge biology and chemistry will provide better simulation of contaminant bioavailability in real soil and aquatic systems, and thus a better basis for correct decisions.

A prerequisite of sound ecosystem management decisions is monitoring of soil and waters that should provide reliable data for solid scientific assessment and its validation, and for supporting and verifying environmental management decisions.

Another emerging task of soil and water screening/monitoring comes from the hazards of eco-terrorism, as these compartments are particularly easily available for an attack, consisting of hazardous chemical, microbiological and biochemical poisoning of water. The specific nature of ecoterrorist's attack requires application of specific techniques, in particular identifying unknown substances and mixtures, type of hazardous action, as well as the extent of acute toxicity and its spatial and temporal extinction. Such topics as advanced "field" and "on-site" analytical techniques, optical sensors and immunochemical-based methods, the miniaturization of instrumentation to permit its use in a field setting for screening/ monitoring is of particular timeliness and urgent need.

In all cases, soil and water monitoring tools should provide an early warning, to prevent, intercept or minimize contamination before the environmental damage proceeds too far.

Minimization of potential of soil and water contamination, also due to use of waste materials such as sewage sludge and other biosolids for soil amendment requires source control of contaminants' emission not only from large industries, but also from small artisan enterprises. For this purpose, simple and inexpensive methods, preferably with use of abundant waste materials, e.g. as efficient and cost-effective sorbents of pollutants may be used. In this way the contaminant loads in sewage sludge from municipal treatment plants could be efficiently reduced, therefore reducing also sewage sludge (biosolids) non-point pollution potential to amended soils. In turn, the opportunities that are brought about by application of many rapidly developing techniques such as permeable reactive barriers for either cutting off the accessibility of reagents causing generation of hazardous contaminants, or reacting with hazardous substances that results in bonding, or decomposing, or transforming them into non-hazardous substances are not yet fully utilized. Many novel solutions might be found in this area, or already known solutions might find a new area of application. Permeable reactive barrier techniques and new sorbents might be equally applicable both for prevention of pollution through control of contaminant generation or transformation them into non-hazardous compounds, but also for efficient remediation of soil and/or water contamination or neutralization of an acute toxicity that might result from accident or eco-terrorism. In all cases, the mechanism of action is of the first priority, but optimization of technical construction/application details are of no lesser importance for assuring efficiency and cost-effectiveness. In the permeable reactive barriers, and other preventive/remediation measures, a number of novel integrated physicochemical and biochemical methods can be used.

The remediation solutions for water/sediments and soil pollution should be focused on non-destructive methods that would allow preservation of soil properties during and after decontamination. One such non-destructive method is phytoremediation, i.e. selection of the best contaminant scavengers applicable in the relevant environment and climatic conditions. for contaminant uptake by plants from water/sediment/soil.

Remediation of soils, water/sediments and groundwater contaminated by complex mixtures of organic pollutants and heavy metals is one the greatest challenges facing environmental restoration. There is therefore a significant demand for environmentally safe and economically feasible technologies for treatment of contaminated ecosystems. Although fundamental and applied studies have provided many novel treatment technologies, we must improve the means to bridge between research and practice, and means to combine different capabilities.

Microbial biotransformations of organic contaminants have been widely observed in soils and sediments, while heavy metal contaminants may be immobilized by the action of metal-reducing bacteria or through sulfide precipitation by sulfate-reducing bacteria. Microbial processes are especially attractive, since biodegradation can result in complete mineralization of organic contaminants or lead to significant reduction in their toxicity. Plant based technologies, phytoremediation, are of interest in the ability of plants sequester contaminants on the one hand, and enhance microbial activities in their root zone, on the other. Accelerated in situ decontamination and/or detoxification through stimulation of desirable microbial and plant-mediated processes can shorten the time frame needed for site remediation, thereby reducing the costs of long term monitoring. Remediation that exploits these natural in situ biological processes, combined with in-place containments, potentially avoids redistribution of the contaminants.

The objective of this book is to summarize and critically assess the current status, development trends and needs of three basic defensive elements that safeguard the quality and environmental safety of soil and water/sediments: early warning monitoring, protection and remediation measures, with particular regard to the viability of methods and technologies, i.e. easiness-to-use, reliability, cost-effectiveness, high efficiency and non-destructive character of remediation that is of particular importance considering the scale of application.

The priority aim of the book is to contribute to the improving maintenance of ecosystems, and specifically to summarize and add to information on how best to protect our soils and aquifers, prevent movement and mobilization of pollutants and how to enhance their degradation and/or immobilization. The remediation solutions for soil pollution are focused on non-destructive methods that would preserve soil properties during and after decontamination.

2. THE SCOPE OF THE BOOK

The book comprises seven major topics that constitute consecutive parts of the book, which contain 38 chapters covering the issues in accordance with its objective and aims:

- Spread and distribution of hazardous chemicals in soils and water as a global problem.

  This part is an introduction into a global character of contaminants of anthropogenic origin and consists of two chapters: one of them discuses the present and the future of contaminants distribution in the whole geosphere. Another is a case study that exemplifies this problem in one developing country where lack of balanced development of industry and environment protection leads to the degradation of ecosystems and has already taken a toll on local economy.
- Fundamental issues concerning fate and behavior of anthropogenic pollutants in soils and water are presented in four state-of the-art chapters showing mechanisms of sorption and bioavailability of metals and anthropogenic organic pollutants in the terrestrial and aquatic environments.
- Advances in chemical and biological techniques for environmental monitoring and predicting are addressed in seven chapters focused on the novel multi-stage sensoristic approach and sensors for environmental monitoring, as well as new techniques, systems and methods for pollutant analysis, simulation of uptake and modeling of transport in environmental compartments.
- Novel physico-chemical techniques of soil and water protection and remediation comprise twelve chapters that present an overview of various treatment techniques and applications, based on permeable reactive or insulation barriers, and on using various sorbents, mostly abundant waste and natural materials or sorbents derived from such materials for metal binding from soils, water and sediments. Other techniques (photolytic, photocatalytic, colloidal/interfacial) for prevention of water contamination by xenobiotic hazardous substances, viruses and other microorganisms are also presented.
- A review of phytoremediation and phytotechnologies and use of different plants – contaminant scavengers for remediation, detoxification, and even for food fortification purposes is discussed in six chapters.
- Assembled plant and microbial technologies for bioremediation and biosensing of soils, sediments and industrial wastes contaminated with heavy metals and organic pollutants is the topic of the subsequent six chapters.

- The final two chapters of the book are devoted to integrated remediation strategies developed for complex management of large-area historically contaminated areas - "megasites". This issue has been exemplified in site-specific strategies applied in three European contaminated sites - port Rotterdam (the Netherlands), Bitterfeld (Germany) and Tarnowskie Gory (Poland).

These, and many other cases of different scale and specificity where contamination identification, monitoring, prevention and remediation is required, show the need of close collaboration of scientists representing different disciplines – chemists, toxicologists, ecotoxicologists, biologists, hydrogeologists, and soil scientists. One of the main messages of this book is thus a call for a better integration between ecology, biology, geology, hydrogeology, chemistry, and soil science: the interaction between these disciplines must become a means for enhanced complementary and mutual contributions to provide optimum practical solutions and technologies for monitoring, prevention and remediation of terrestrial and aquatic environmental contamination.

The contributors to the book are internationally recognized experts, and present both state-of-the art and novel approaches in the above fields, that makes the book unique with respect to the scope. The book is addressed to the vide range of professionals and decision-makers involved in the soil and water pollution monitoring, prevention and remediation issues, as well as to academic teachers and university students.

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The idea of this book has arisen from the mutual experience, contacts and discussions of specialists from different countries involved in the problems of anthropogenic contamination of the terrestrial and aquatic environment, bioavailability, risk assessment, screening, monitoring and prognosis of contaminant migration, prevention and non-destructive remediation measures. The editors, on behalf of the authors and themselves, express their deep gratitude to NATO Science Affairs Division for the financial support, which made it feasible to organize a meeting of 49 invited prominent specialists within the NATO Advanced Research Workshop (NATO-ARW) entitled "Viable Methods of Soil and Water Pollution Monitoring, Protection and Remediation" held from June 26 to July 1, 2005 in Krakow, Poland, and to publish this book that is an outcome of this remarkable meeting.

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Introduction: Spread and Distribution of Hazardous Chemicals in Soils and Water – A Global Problem

1.1. Diagnosis and prognosis of the distribution of contaminants in the geosphere
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1.2. Persistent organic pollutants in Egypt - an overview
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DIAGNOSIS AND PROGNOSIS OF THE DISTRIBUTION OF CONTAMINANTS IN THE GEOSPHERE

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Abstract: Only in the late 1970's did the industrialized countries begin to comprehend the enormity of the damage brought about by pollution of the geosphere. Although the harm caused by the use of DDT and other organochlorine pesticides was already noted by Rachel Carson in her 1962 seminal book 'Silent Spring', the first major institutional move to stop pollution and reclaim polluted land was in 1978, when the Love Canal neighborhood was evacuated and the U.S. Superfund program initiated. The geosphere contains huge quantities of pollutants deposited in the past and more are still emitted today. This time-bomb threatens to release its toxic content into groundwater, surface water and the atmosphere and to contaminate the biota. Although the distribution of pollutants in the geosphere is hard to estimate since there is a continuous exchange of pollutants between the geosphere, biosphere, atmosphere and hydrosphere and pollutants undergo a myriad of both chemical and physical transformations, the state of the geosphere is undoubtedly worrisome. It is estimated that about 500,000 km^2 of the pre-2002 EU (16% of the land area) and about 35% of the land area of the countries that were then candidates for joining the EU are polluted to some degree. The prognosis is, however, better. Throughout the world, major efforts are being made to reduce pollution and remediate contaminated areas, and indeed signs of recovery are evident. In the U.S, for example, both the total amount of pesticides used and the quantity applied per unit area is declining steeply. The amount of herbicides added per unit area of corn, decreased by 25% and of insecticides by 60% in the decade between 1991 and 2001.

Key words: geosphere; global contaminants distribution; leading contaminants; organic pollutants; trace metals

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1. INTRODUCTION

The pollution of the geosphere is continuing relentlessly ever since the beginning of the Industrial Revolution, but only in the 1970s did decision makers begin to comprehend the severity of the damage it caused. One of the first to raise the alarm was Rachel Carson, who already in 1962, in her book Silent Spring, warned of the harm caused by the use of pesticides and in particular the persistent DDT and other organochlorine compounds. It took, however, more than a decade for authorities anywhere to take action. The first major institutional move to remediate polluted land and to stop the deterioration in soil and water quality which came perilously close to the point of no-return, was in 1978, when the Love Canal neighborhood in the city of Niagara Falls, New York was evacuated and the Superfund program was initiated. A ditch at the site where construction of a never-completed canal has begun by William T. Love was turned into a disposal site for municipal and industrial chemical waste. In 1953, the company that then owned and operated the site, covered the ditch with earth and sold it to the city of Niagara Falls for one dollar. Twenty five years after the Love Canal ceased to serve as a dump, over 80 compounds, 11 of them presumed to be carcinogenic, percolated upward, after their containers rotted and released their contents to the surface in yards and basements, including that of a public school built on the banks of the hazardous waste-filled ditch.

In response to the Love Canal environmental catastrophe, the U.S. Congress established a series of acts aimed at remediating polluted lands, halting ongoing pollution of the geosphere and preventing future pollution. U.S. EPA (United States Environmental Protection Agency) that came into being only eight years earlier was responsible for carrying out its mandates. The first act was named CERCLA (Comprehensive Environmental Response, Compensation and Liability Act) and was followed by SARA - The Superfund Amendments and Reauthorization Act. These acts established, for the first time anywhere, a government trust fund devoted to the remediation of polluted sites, the size of which was US$8.5 billion. It took Europe a few more years to join the fight against the pollution of the geosphere. The earliest inventories of POPs (persistent organic pollutants) were compiled in Europe only in the 1980s.

2. THE GLOBAL DISTRIBUTION OF POLLUTANTS IN THE GEOSPHERE

Despite the numerous measurements taken throughout the globe, an exact mapping of the global distribution of major pollutants is not possible. The distribution of pollutants in the geosphere is hard to estimate since it is ever changing. There is a continuous exchange of pollutants between the geosphere, biosphere, atmosphere and hydrosphere and pollutants undergo a myriad of biological, chemical and physical transformations. The complexity of the dynamics of pollutants distribution is exemplified in Figure 1 (Fernandez and Grimalt, 2003). Notwithstanding the uncertainty in defining its precise condition, the state of the geosphere is undoubtedly worrisome.

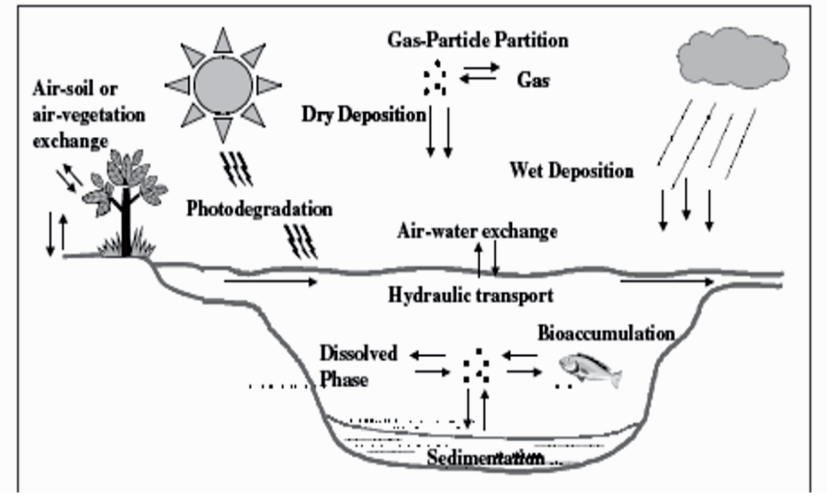

Figure 1. Main environmental processes governing long-range transport of pollutants (adapted from Fernandez and Grimalt, 2003).

It is estimated that 16% of the area of the countries that belonged to the European Union in 2002 (about 500,000 km^2) and 35% of the land area of the countries that were candidates for joining the EU at that time are polluted to some degree. The European Environment Agency estimated that there are about 1,500,000 sites that require soil remediation throughout the EU (Pollution Online, 2002). The precise number of polluted sites depends strongly on the definition of the maximum allowed levels of pollutant concentrations and on the thoroughness with which pollution is sought out. Thus, in the Netherlands, 350 sites were declared as requiring remediation in 1981, while in 1995 – 300,000 sites were designated as requiring remediation at a cost of 13 billion Euros. It is not likely that within a fifteen year period pollution spread to such an extent. It is much more likely that the immense increase in the number of polluted sites arose from the tightening of regulations regarding soil pollution and from soil analyses becoming mandatory or at least voluntarily performed in more cases.

2.1 Models

The fate of pollutants in the geosphere is not easily quantified. They may undergo, each pollutant to a different extent and through different pathways, numerous processes such as sorption, degradation, plant uptake, vertical transport and volatilization, and the interrelation between these processes is complex and non-linear. The major tool for overcoming the enormity of the task of defining the global distribution of pollutants is modeling. Predictive models are intended to forecast the future fate and distribution of pollutants from presently available data (or define the present state from past-collected data). However, even in the ideal case, models produce a good approximation of the most likely future scenario (or scenarios) but not yield an exact determination.

The limited predictive capacity of models is an unavoidable outcome of a few factors. Firstly, the measured data which serves as input for any given model's calculations is always incomplete. Data being collected by different research groups are likely to be measured at different spatial and temporal scales, making the integration of these data to a uniform input base rather complicated. Secondly, our understanding of the processes that pollutants undergo in the different compartments of the geosphere and of the myriad of interactions with biotic and abiotic entities is incomplete. This lack of complete knowledge results in the adoption of various approximations, (namely the construction of different models), each being suitable to a limited set of uses or conditions. Important processes which are not fully understood are often described by their effects, or in other words, the model employs empirical parameters. Such parametrization further limits the scope of the model, making its predictions less accurate as the environmental conditions for which the predictions were made become less similar to those of the systems in which data used for the definition of the model's parameters were collected. Different modeling approaches lead to predictions that at times vary significantly from each other. Finally, with all the breathtaking advances in computing capacity, present day computer power is still inadequate for the incorporation of all relevant interactions and processes in a single executable model, thus necessitating the adoption of rather crude approximations.

Nevertheless, a careful use of models, performed by personnel aware of the limitation of the specific model they chose to utilize, can give a reliable estimate of the present and future distribution of pollutants in the geosphere. Correctly used models serve as cost-effective tools for environmental impact assessments and make it possible to regulate the use of agricultural chemicals, identify areas which are potentially vulnerable to surface and groundwater contamination from non-point source pollution, or support ecosystem restoration goals. Environmental simulation models may serve as relatively inexpensive alternatives to costly field monitoring strategies. Even if modeling cannot yet completely substitute for laboratory or field measurements, it can serve as a complimentary assessment method, allowing more efficient use of data obtained from field and laboratory studies.

For example, according to EC Directive 91/414/EEC, the European Union requires information on Predicted Environmental Concentrations (PECs) in soil, groundwater, surface water and air of bioactive substances and their toxicologically and environmentally significant metabolites as supporting data in applications for authorization of the use of plant protection products. PEC values are used to determine whether additional studies (e.g. field or lysimeter studies) are required to assess the mobility of the product in soil and its potential for leaching into groundwater. Validated models are indispensable tools for predicting environmental concentrations in soil, groundwater and surface water. The EU is using a modification of the EPA version of the PRZM model developed by an industry/academic/EPA consortium named the Forum for International Co-ordination of Pesticide Fate Models and their Use. It goes by the acronym FOCUS (1997). FOCUS has developed standardized scenarios, intended to cover a range of conditions simulating those existing throughout the EU, for performing first-tier assessments of the exposure of groundwater and surface water to organic pollutants. These scenarios are due to become an essential part of the process of authorization of pesticides for use in the EU.

In the Netherlands, the model PESTRAS (Pesticide Transport Assessment) model was developed for use in assessing the fate and behavior of organic pollutants in soil. Processes like adsorption-desorption kinetics, the formation and behavior of reaction products, vapor diffusion in the gas phase and the volatilization of pesticide from the soil surface are included in that model, which is coupled to a model for soil water flow. Later on, the PEARL (Pesticide Emission Assessment at Regional and Local scales) model, simulating the behavior of pesticides in soils, was developed. Important features of that model are: (i) object oriented design, (ii) model and data are accessed through a user-friendly Graphical User Interface, (iii) data and scenarios are stored in a relational database, and (iv) easy link with external programs, such as Geographical Information Systems and inverse modeling tools. The PEARL model is now the official tool in Dutch pesticide registration procedures. It is a one-dimensional, multi-layer model that describes the fate of a pesticide and its relevant transformation products in the soil-plant system and considers the processes of transient state soil water flow, potential evapotranspiration, interception of water, water uptake by plant roots, evaporation of water from the soil surface, lateral discharge, heat flow, pesticide application, dissipation of pesticide from the crop canopy, convective and dispersive transport of pesticide in the liquid phase, diffusion of pesticide through the gas and liquid phases, equilibrium sorption and non-equilibrium sorption, first-order transformation kinetics, uptake of pesticide by plant roots, and volatilization of pesticide at the soil surface.

Environmental-fate and transport models vary in their complexity from simple indexing models to highly sophisticated stochastic models, which take into account a large number of relevant parameters and the dependence of their value on local conditions. Among the many index models that exist, noteworthy are LEACH and VOLAT (Laskowski et al., 1982), travel time(Jury et al., 1984), attenuation factor (AF) and retardation factor (RF) (Rao et al., 1985), mobility and degradation index (MDI) (Mahmood and Sims, 1986), leaching potential index (LPI) (Meeks and Dean, 1990) and mass-fraction models (Zee and Boesten, 1991; Beltman et al., 1995). These models are widely used for ranking the pollution hazard of chemicals, and in combination with geographical information systems (GIS) they can be used for regional-scale preliminary assessments of groundwater vulnerability to nonpoint-source pollutants (Khan and Liang, 1989; Hoogeweg and Hornsby, 1995; Hollis, 1988-1990; Loague et al., 1996). Kleveno et al. (1992) compared the AF model with the considerably more detailed PRZM model (Carsel et al., 1984) and found that the AF compared well to PRZM in estimating the relative mobility of pesticides in heterogeneous soils and under conditions of variable recharge. The latter model is the only significant pesticide model acceptable for regulatory purposes in the US. Nearly all geospheric transport models are deterministic and employ the one-dimensional flow approximation for flow in the unsaturated zone. Such models generally do not consider horizontal flow and do not produce a full 3-dimensional description of the flow process. Description of numerous models can be found in Jury and Ghodrati (1989), Jarvis et al. (1995) and Vanclooster et al. (2000).

2.2 Leading Contaminanats

Pollutants that are of more than local interest as far as contamination of the geosphere is concerned, must possess a number of characteristics, the more important of which are toxicity, persistence and a capacity to disperse, which in turn depends on the pollutant’s leachability (or water solubility), volatility and tendency to sorb on particulate matter that has the potential to be air-borne.

The contaminants that have the highest impact on the geosphere may be classified as follows:

Trace elements – Concerning the global pollution of the geosphere, probably the most significant trace elements are the metals Hg (mercury), Cd (cadmium) and Pb (lead).

Organohalogens – These are organic substances containing halogens, especially chlorine and to a lesser extent bromine or fluorine. The more common groups in this family of compounds are:

- Halogenated aliphatic hydrocarbons. Probably the most important member of this group is TCE (trichloroetane or trichloroethylene) which is used heavily as a solvent or a degreasing agent in various industries and in dry cleaning.
- Halogenated aromatic hydrocarbons, such as dioxins. Dioxins are a group of several hundred substances that belong to two related sets of compounds: chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs).
- Chlorinated phenolic compounds, such as polychlorinated biphenyls (PCBs; Figure 2).
- Organohalogenateded pesticides, such as aldrin, dieldrin, andrin and DDT.

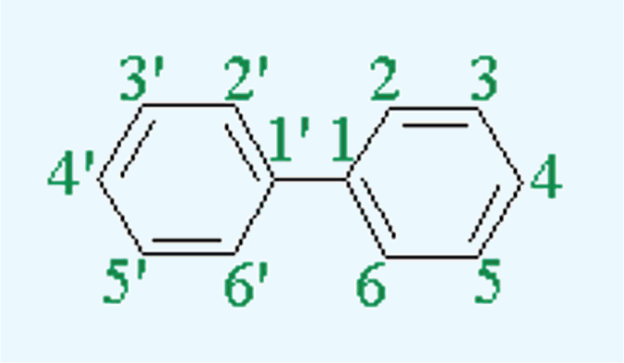

Figure 2. Schematic representation of PCBs.

Polyaromatic hydrocarbons (PAHs) - This is a group of over 100 organic compounds composed of several fused benzene rings (e.g., anthracene and pyrene). Some PAHs are persistent and carcinogenic and they are formed during the incomplete combustion of organic substances such as coal, oil, gas or garbage.

The United Nations, through its Environmental Program (UNEP), drafted the Stockholm Convention on persistent organic pollutants (POPs) which was adopted in 2001. The POPs listed in that convention as being of major environmental concern are given in Table 1.

Table 1. POP list adopted by the Stockholm Convention on persistent pollutants.

Aldrin	Heptachlor
Chlordane	Hexabromobiphenyl
Chlordecone	Hexachlorobenzene
DDT	Hexachlorocyclohexanes
Dieldrin	Mirex
Dioxins	PCBs
Endrin	PAHs
Furans	Toxaphene

2.3 Global Cycles of Persistent Pollutants

The global distribution of persistent pollutants, both POPs and toxic trace elements is not stationary. Neither is it determined by a process in which pollutants are added at a given location by man-made activity on the one hand and removed by natural processes such as leaching, evaporation or degradation on the other. Pollutants rather undergo a continuous cycle of redistribution, while an anthropogenic, freshly produced fraction may at any time be added into that cycle and another fraction is removed by both natural and anthropogenic processes.

The global spread of persistent organic pollutants has become one of the main environmental problems of the last decade (Fernandez and Grimalt, 2003). POPs can reach remote locations, far away from the source of pollution by a number of mechanisms. These include transport through the atmosphere in the gaseous state or in aerosols, and through the hydrosphere and the geosphere, in solution or attached to suspended particles, (for example, due to erosion). The presence of pollutants in remote areas is to a large extent due to transport in the atmosphere as a gas and hence, the more volatile the compound, the higher the probability for it to be found further away from its source. This phenomenon is sometimes termed the global distillation effect. The concentration of some POPs in polar regions and at high altitudes, where low temperatures result in condensation and consequently deposition, was reported to reach levels exceeding human consumption guidelines (AMAP, 1998).

Following is a brief discussion concerning some of the more important POPs. Dioxins are considered to be the most highly toxic group of xenobiotic compound. The tetrachloro dioxin congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; Figure 3) is harmful in food and water at sub-ppt levels, and in fact, the term dioxin is used at times to refer to this congener. CDDs and CDFs are not commercial products, but are rather inadvertent byproducts of human activities such as industrial or municipal waste incineration or burning fuels (e.g., wood, coal or oil). Other activities, including chlorine bleaching of pulp and paper and certain processes employed in the chemical industry can also generate small quantities of dioxins. So can cigarette smoking. Natural processes, for example lightning-induced forest fires, can produce CDDs and CDFs as well and accordingly, dioxins were detected in the geosphere in locations where anthropogenic products are not likely to be present. Thus, traces of dioxins at the ppt level were found in buried clay deposits, both in Europe and in the U.S. Recent assessments confirm that anthropogenic emissions are the major source of dioxin released to the environment, but there is insufficient data to quantify the contribution of natural sources to the dioxin content in the geosphere.

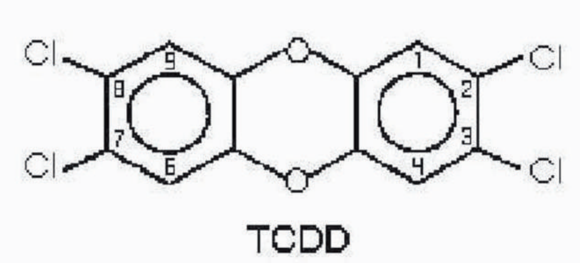

Figure 3. Structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

The global cycle of dioxins consists of suspension and formation of wind-born aerosols of particles (or droplets) that either contain dioxins or have them sorbed on their surfaces, and of evaporation of dioxins from soils and surface waters to the atmosphere. From there, redeposition back to land and surface water occurs. Adsorption to sediments and bioaccumulation are the primary removal mechanisms from surface water, until resuspension occurs again.

PCBs are probably the most important persistent organic pollutants. These compounds are dielectric liquids, resistant to both chemical and biological decomposition. Their major use is in transformers and other electric components. Due to their widespread use and persistence, PCBs were detected throughout the globe, from livers of seals in the Antarctic to mother's milk in Europe. Deleterious effects in humans attributed to PCB uptake were detected, among other locations, in Taiwan and Japan. These effects included malformations in babies whose mothers were exposed to PCBs (Gladen et al., 2003). Their relatively low solubility in water provides a modicum of protection against acute poisoning (McFarland and Clarke, 1989), but the likelihood of long term accumulation of PCBs and their ubiquitous nature, make this family of compounds probably more hazardous than the considerably more toxic dioxins.

Studies on the distribution of PCBs in soils and the sub-soil (as well as in tree leaves) that were conducted in England, Scotland and Italy (Cousins et al., 1999), confirmed the widespread occurrence of PCBs in the geosphere. The work performed in the British Isles focused on sites in agricultural research farms far removed from potential PCB sources, thus ensuring that the PCB detected originated from aeolian deposition. In Italy, samples were taken in locations of varying proximity to PCB sources, including two remote Alpine valleys. As expected, there was a gradient in PCB concentration in soils, the concentration declining as the distance from PCB sources increased. However, even in the more remote locations both in Italy and the U.K., PCBs were detected. At all these remote locations, PCBs concentrations were similar, even if relatively low. This observation suggests the existence of a mechanism, most likely wind born, for a more or less uniform spread of PCBs throughout much of the globe, with peaks near contaminant sources. The above studies indicated that the leaching of PCBs downward in the unsaturated zone is rather slow, PCB concentrations at the soils' surface being a few orders of magnitude higher than at 20 cm depth. A similar vertical distribution was found in soils of Norwegian forests (Krauss et al., 2000).

Another pathway of PCBs dissemination was demonstrated in studies conducted in France and Canada. Domestic sewage was shown to contain considerable concentrations of PCBs, much of it apparently originating from aeolian deposition. Secondary treatment removed much of the PCBs' content, totally eliminating many of the congeners, especially the more hydrophobic ones. However, much of the removed PCB content remained in the sludge, sometimes resulting in a high enough concentration of PCBs to be of ecological and human-health significance (Blanchard et al., 2001; Pham and Proulx, 1997). Thus, irrigation with effluents or application of sewage sludges to soils, a common practice in many countries, may constitute a significant source of PCBs reaching the geosphere.

In summary, the major mechanism for PCBs mobility is a cyclical evaporation from soil and water surfaces and winds lifting PCBs into the air along with water vapor and dust, eventually redepositing them with rain and snow, condensed from the gas phase or adsorbed to settling particles. Because of their considerable environmental mobility, once emitted to the environment PCBs enter a global pool in which they remain susceptible to redistribution (Iwata et al., 1995; Tanabe, 1998). As a result of this high mobility and their substantial persistence, the concentrations of the PCBs tend to equalize throughout the globe (Tanabe et al., 1994). Accordingly, monitoring data indicate that the polar regions may become a sink for PCBs (Muir et al., 1992).

Munthe and Palm (2003) composed the final report on The Atmospheric Cycling of Mercury and Persistent Organic Pollutants, submitted to EUROTRAC-2 (A EUREKA Environmental Project). Hg was chosen for study since, of all the trace elements, it is of the highest global concern. It is toxic and its high volatility and the large number of species in which it is found in the environment dictate a world-wide distribution in all environmental compartments, including the hydrosphere, atmosphere and geosphere. Being an element and hence stable, Hg poses a long term risk to the global environment.

The major anthropogenic sources of mercury are emissions from combustion. Electric power plants, non-ferrous metal production plants, steel and pig iron foundries, cement kilns and waste disposal sites (especially waste incinerators) are prominent examples of mercury emission sources. In addition, considerable amounts of Hg are emitted from chlor-alkali plants, gold production installations, battery production plants and manufacturing of measuring and control instruments, electrical lighting, wiring devices, and electrical switches. About three-quarters of the total Hg emissions were attributed to combustion of fossil fuels, specifically of coal. Figure 4 summarizes the anthropogenic emission of mercury in 1995. Asia, and especially Japan, China, South and North Korea and India, is by far the largest source of mercury emission, China contributing more than 25% to the total global emissions.

Yet, Europe and South Africa are also significant contributors of mercury to the environment.

The environmental mobility of mercury is strongly dependent on the chemical speciation and physical mode (gas state, adsorbed etc.) in which the element is present. The major chemical form of mercury emitted to the atmosphere is gaseous elemental mercury, contributing around 50% of the total emissions. Bivalent mercury that exists as different nonionic organic species and as a cation is also commonly emitted into the environment. Atmospheric (and to a lesser extent effluent) emission of Hg sorbed on particulate matter contributes about 10% of the total mercury emitted. Identifying the chemical speciation of mercury emitted into the environment from a given source is crucial for the assessment of environmental and human health risks as well as for the estimation of transport and fate of the emitted mercury.

The exceptional volatility of mercury makes it the leading metal as far as global spread is concerned, but other metals such as Cd and Pb, are of major environmental concern. Since they are less volatile, these metals display a higher tendency to concentrate in areas that are relatively close to their source. The less volatile metals do spread through the environment, sorbed or embedded in particulate matter.

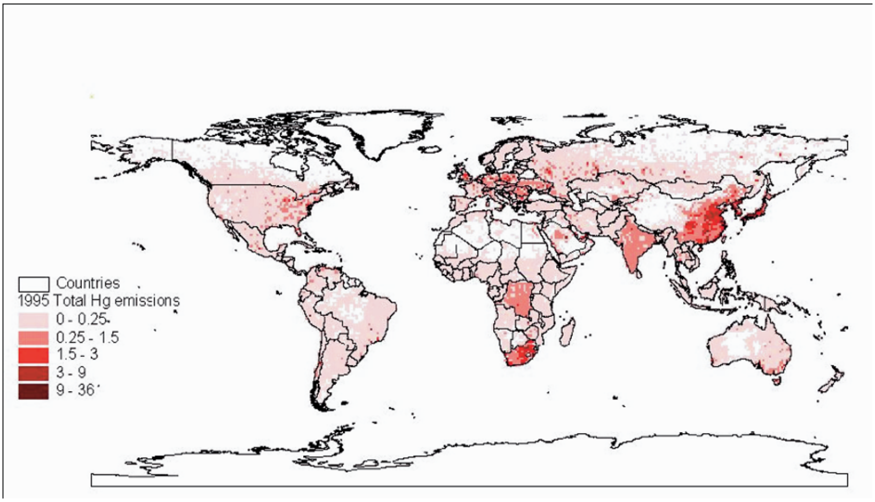

Figure 4. Anthropogenic emissions of total mercury (tons) in 1995 (Munthe and Palm, 2003).

Yet, the fact that in the case of Hg, only 10% of the atmospheric emission is in particulate form, indicates that atmospheric deposition of metals other than Hg is a less important source of geospheric pollution in locations that are situated far away from the pollution source.

Metals can be transported vertically as well as horizontally in the liquid phase, both as solutes and attached to suspended particles. The transport of metal ions, especially downward through the unsaturated zone, is enhanced by the formation of neutral and anionic complexes with various ligands, prominent among which are the soluble components of the organic fraction of soils or of soil additives such as sludge (Mingelgrin and Biggar, 1986; Vulkan et al., 2002). Figure 5 presents a paper electrophoresis chromatogram of the water soluble Cu species extracted from a sewage sludge. This chromatogram demonstrates the abundance of water soluble copper species present, most of them neutral or negatively charged.

Not only is enhanced transport via attachment to soluble species important to the downward movement of metal cations, but it is also relevant to the distribution in the geosphere of organic contaminants. Figure 6 demonstrates that the vertical transport of the pesticide atrazine was strongly enhanced by irrigation with an effluent. Sewage effluents are richer in potential carriers (e.g., dissolved organic matter, including detergents) than the high quality water used as the reference irrigation water in the study (Graber et al., 1995) in which this data was generated.

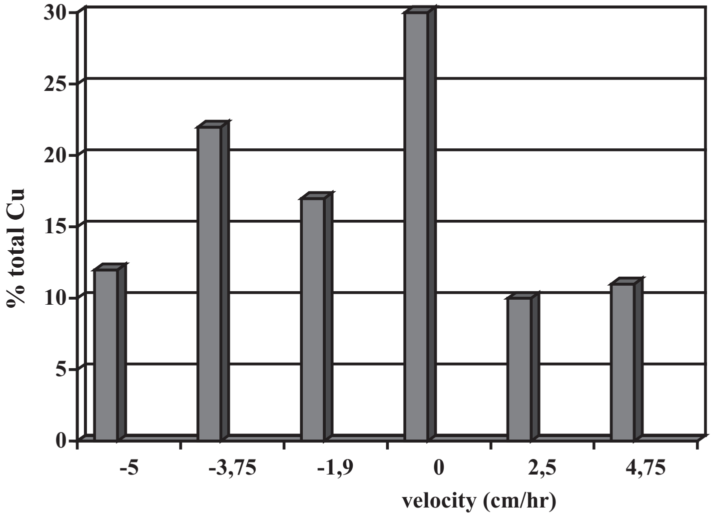

Figure 5. Paper electrophoresis chromatogram of copper species in a Davis sludge saturation extract (Mingelgrin and Biggar, 1986).

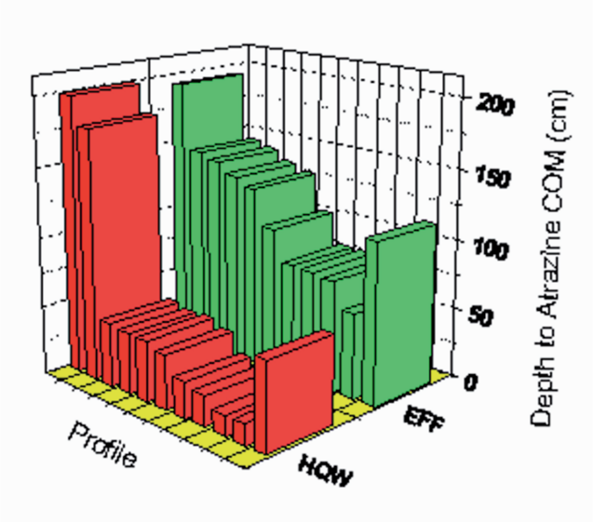

Figure 6. Atrazine transport under irrigation with sewage effluent (EFF) and high quality water (HQW) (Graber et al., 1995). COM refers to center of mass.

2.4 Case Studies

Meijer and co-workers (2003) conducted a survey on the global distribution of PCBs and HCB (hexachlorobenzene) in the soil's upper horizon. That team measured the concentrations of the investigated pollutants in 191 surface (0-5 cm) soils. Differences of up to 4 orders of magnitude were found for PCBs' concentrations between sites. The lowest and highest PCB concentrations (26 and 97,000 pg/g dw) were found in samples from Greenland and from mainland Europe (France, Germany, Poland), respectively. The authors estimated the total global load of PCBs in soils to be 21,000 ton.

PCB concentrations in soils were strongly influenced by proximity to the source of the pollution. The concentrations also increased with soil organic matter content, since PCBs, being rather hydrophobic tend to sorb to, and thus accumulate in, the organic fraction of the soil. Most (>80%) of the estimated PCB load in soils is found in the major "global source region", namely in the northern hemisphere at temperate latitudes (30-60 degrees N), or in the organic matter-rich soils just north of that region. The distribution of PCB consumption throughout the globe is given in Figure 7 and the good correlation between the concentration of PCBs in soils and between their consumption is evident.

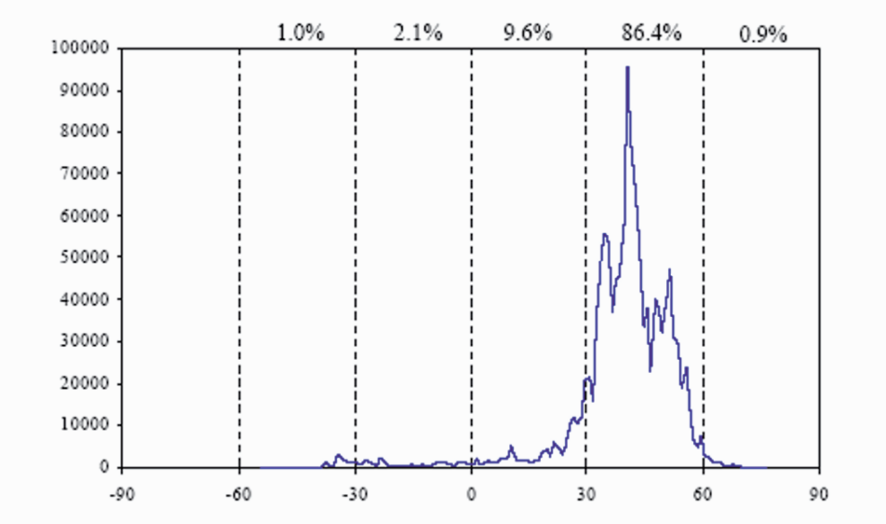

Figure 7. Estimated cumulative consumption of PCBs by latitude (in tons/degree) (Munthe and Palm, 2003).

The findings of the above study indicate that a gradient in PCBs concentration has developed throughout the globe, the concentration declining as the distance from the pollution source increases. Yet, significant concentrations of the pollutants were found at remote sites.

Thus, this comprehensive study confirmed the results of other investigations concerning the global distribution of PCBs (e.g., Section 2.3) that suggest a tendency of the concentration of PCBs to become similar throughout the globe, with the exception of peaks in concentration in the vicinity of the sources of pollution.

Accordingly, recent studies demonstrated that organochlorinated pollutants, including PCBs and organohalogenateded pesticides, are found in measurable concentrations all over the globe, even in areas far from the sources of pollution. For example, although they inhabit a remote location, albatrosses in the Midway Islands contained DDT, PCBs and dioxins in the adult birds, chicks and eggs at concentrations nearly as high as the concentrations found in bald eagles in the North American Great Lakes (e.g., Auman et al., 1997).

Fernandez and Grimalt, 2003, tried to define the global distribution of POPs, while minimizing the influence of local pollution foci. To that end they studied remote sites, specifically high altitude mountain regions. These authors measured the concentration of numerous POPs in water, soil, air and lake sediments in selected locations throughout Europe and the Tenerife Island. PAH concentrations in the sediments were particularly high in Eastern Europe (Tatra Mountains). In all other studied locations, the concentrations were similar, yet much lower then in the Tatra Mountains (Figure 8). Concentrations in soils and air samples showed a similar trend, demonstrating that air-borne particulate matter and the air gas phase are the main transport media for PAHs. Accordingly, atmospheric deposition is the main mechanism of geospheric pollution far enough from the source of pollution. In contrast, the concentrations of the organochlorinated compounds measured in that study did not display an orderly geographic distribution or a distinct relationship between their concentrations in the different environmental compartments (Figure 9). Fernandez and Grimalt (2003) did, however, discern an altitude-dependent concentration gradient for the organochlorinated compounds which was more evident for the less volatile PCBs, suggesting a condensation phenomenon from the gas phase.

2.5 Prognosis of the State of the Geosphere

Pollutants have been reaching the geosphere for many years now, both directly as a result of local anthropogenic activity and after being carried, at

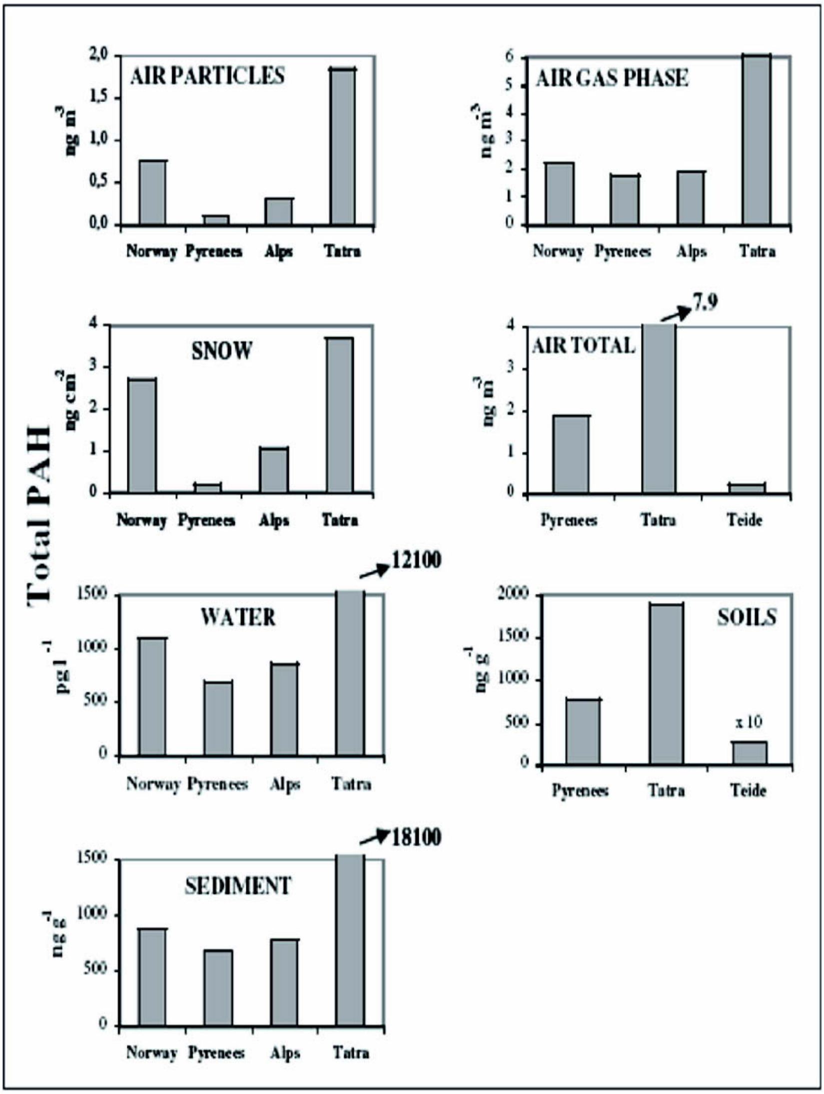

Figure 8. Total PAH levels (sum of 23 compounds from fluorene to coronene) at selected high altitude regions (Fernandez and Grimalt, 2003).

times for long distances, through the hydrosphere or the atmosphere. The geosphere, and in particular its upper horizons, has thus turned into a depository of a myriad of contaminants and their degradation products. Consequently, the state of the geosphere, as far as its load of pollutants and the resultant damage to the ecology and to human health, is bad and in numerous locations pollution has reached the level of a veritable environmental disaster. The prognosis for the future quality of the geosphere is, however, guardedly good. Throughout the world, major efforts are being

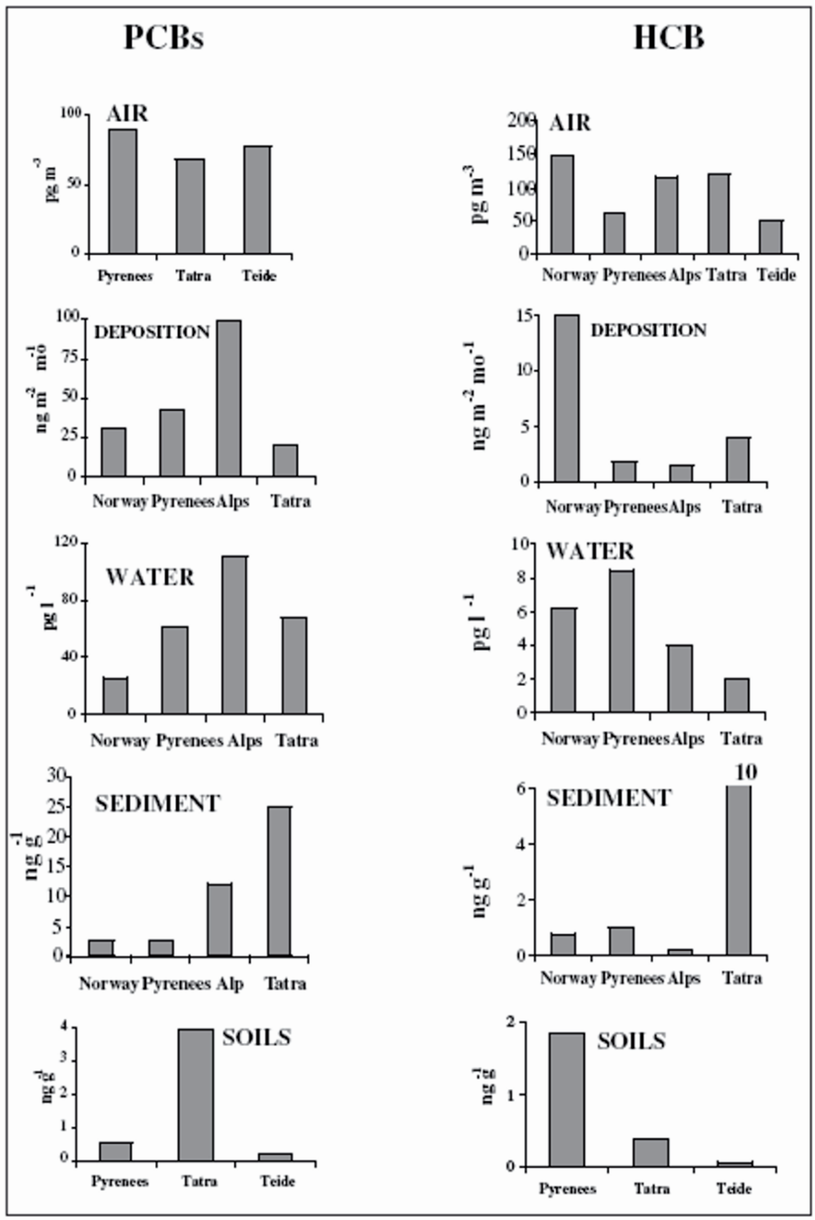

Figure 9. Concentrations of PCBs (sum of 7 congeners) and HCB at selected high altitude regions (Fernandez and Grimalt, 2003).

made to reduce pollution and to remediate already contaminated areas. Sampling and analytical procedures both ex- and in-situ have greatly improved in recent years. One interesting example (Terry et al., 2001) is the development of a technology to detect the elusive DNAPLs with the aid of hydrophobic dyes. Similarly, remediation protocols are becoming more technically efficient and economically affordable, thus making it feasible to remediate a wider range of polluted areas. And indeed, signs of recovery are evident.

Various indicators of environmental pollution point at a reduction in the extent of contamination of the geosphere. This turning of the tide is particularly evident in the more developed countries, but it also holds true on a global scale. Dioxins, for example, are only one of several families of contaminants for which fish advisories have been withdrawn by several states in the U.S. One reason for this is that many pulp and paper mills have changed their bleach kraft processes in a way that reduced the levels of dioxins in their effluent. The number of advisories for dioxins that were issued in the U.S., fluctuated between 1993 and 1997, with the highest number of advisories (65) being in effect in 1997. Since then, the number of advisories declined. Likewise, federal efforts to enforce regulations addressing PBTs (persistent, bioaccumulative, and toxic compounds), have yielded a considerable reduction in PBT emissions. The U.S. EPA estimated that in 1999 alone, the amount of PCB waste released in the U.S. was reduced by 129 million pounds as compared to the previous year and the amount of PAHs released declined by four million pounds (EPA, 2000). The amount of soils contaminated by dioxin, lead or arsenic was reduced by 573 million pounds in that year (EPA, 2000).

The amount of persistent pesticides used globally, and in particular of pesticides that are highly toxic to non-target organisms, is on the decline. New pesticides are more effective against their target pests and at the same time less harmful to other organisms. In the U.S., both the total amount of pesticides used and the quantity used per unit cultivated area are being reduced sharply. In corn production, for example, the amount of herbicides added per unit area declined by 25% and the amount of insecticides by 60% between 1991 and 2001. The fraction of pesticides listed in the United States that is defined as highly toxic to mammals, declined by more than 50%. Current trends in plant protection practices further reduce the risk pesticides pose to the environment. These trends include the development of precision agricultural procedures, whereby sensors such as remote sensing devices, enable the optimization of application time and dose at a high spacial resolution. The broader acceptance of integrated pest management, in which biological and other non-chemical plant protection methods are integrated into the pest control regime, and the fast expansion of organic agriculture are also welcome trends from the environmental point of view.

The above optimistic prognosis should not divert our attention from local foci of pollution still active. The high cost of adopting pollution-preventing measures, led many firms and municipalities to resist the efforts of environmental agencies to impose the cessation of emission of pollutants. This is true even in countries in which awareness of the harm done by the emitted pollutants is very high, let alone less environmentally conscious countries. Likewise, the cost of soil remediation, which despite of recent advances is still very high, leads many firms to disavow their responsibility to past and present contamination and to fail to disclose emissions that would require cleanup.

Countries that are experiencing an intensive process of industrialization tend to neglect, in the midst of that process, environmental concerns. Strict international supervision is at times necessary to keep these sources of pollution to a minimum. A case in point is China, in which environmental pollution has reached a critical level in some places. Yet, the fact that the Chinese government has declared already in 2002 the launching of a massive 700 billion yuan ($84 billion), 5-year environmental protection plan to combat the worsening pollution, suggests that this large country is also approaching the stage of receding environmental pollution.

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PERSISTENT ORGANIC POLLUTANTS IN EGYPT - AN OVERVIEW

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Abstract: Over the past few decades, scientists around the world have revealed the sinister impacts that persistent organic pollutants (POPs) could inflect on man and his environment. Growing interest in monitoring and managing POPs was building up at a worldwide level and Egypt was no exception. Egypt has ratified the Stockholm Convention for controlling the emission of POPs and had its National Implementation Plan (NIP) in accordance with other signatory countries. On the other hand, a set of environmental measures was adopted for environmental protection aiming among other things to minimize the emission of POPs in different environmental segments. A prominent component of the NIP was the preparation of an inventory of POPs that main industries and other activities produce. POPs use in Egypt was mainly limited to organochlorine and cyclodiene pesticides, used extensively to protect cash crops. With Egypt catching up with industry–based economy, numerous industries were established with the likelihood of using, emitting a wide array of chemicals that include POPs. Residues of some POPs, especially DDT and other pesticides were monitored in a variety of environmental matrices that include marine, freshwater, humans, food and many others. Monitoring studies of PCDD and PCDF were comparatively less frequent because of the special techniques required. The present review sheds some light on some of the prevailing conditions of POPs, their emission, management and institutional aspects. Residues of some POPs in some Egyptian environmental segments are reported.

Key words: persistent organic pollutants; Egypt; residue level; environment

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1. INTRODUCTION

Time has proven that the revelation Rachel Carson made in her memorable book “Silent Spring” was only a prelude to a stormy future in which xenobiotic pollutants would impact human life to an unprecedented degree. Today, the contamination from manmade chemicals is a pervasive global problem urgently demanding a global solution.

Between the vast array of contaminants that plague the environment, persistent organic pollutants POPs represent the most potentially hazardous contaminants. Persistent Organic Pollutants (POPs) are synthetic chemical substances with unique and harmful characteristics; they include highly toxic pesticides such as DDT; industrial chemicals such as PCBs; and unintended by-products of industrial and incineration processes such as dioxins and furans. In addition Polycyclic Aromatic Hydrocarbons (PAH) are included in POPs due to their physicochemical properties and their behaviour in the environment. PAHs are by products of incomplete combustion of organic matter at high temperature and are usually on the surface of solid particles; these compounds are transported and distributed into all environmental systems, including surface and even groundwater, via dry or wet deposition. The US EPA has established 16 PAHs as priority pollutants, with benzo-a-pyrene selected as a gross indicator for PAHs.

Persistent organic pollutants share some common characteristics that include high toxicity, persistence, and special affinity to fat. Due to their semivolatile nature, persistent organic pollutants are transported over long distances in both air and water, with temperature playing a key role. The long range transport of persistent organic pollutants has been described as a combination of atmospheric and sea current transport, or as a global distillation process. Persistent, semivolatile organic pollutants are transported over different distances prior to their deposition onto the sea surface, sediment, or soil, depending on such physical properties as vapour pressure and ambient temperature. Furthermore persistent organic pollutants will be re-evaporated into the atmosphere and undergo further atmospheric transport to the Artic region after their deposition depending on the weather conditions and surrounding temperature. This process is known as the grasshopper effect. Such long range transport behaviour of these compounds has become a major political and scientific issue (Beyer and Matthies, 2001).

2. TOXICITY

Persistent organic pollutants are ubiquitous, their levels tend to increase in species at the top of the food chain, such as eagles, polar bears, killer whales and human beings. POPs will remain in the environment for a long time even if all new sources will be immediately eliminated as the rate of their degradation is very low. There is evidence that a lot of people worldwide may now carry enough POPs in their body fat to cause serious health problems, including reproductive and developmental problems, cancer, endocrine and immune system disruption, abnormal behaviour, and neurological problems. Furthermore, developing embryos are most sensitive to the harmful results of POPs.

All the targeted POPs have been identified as endocrine disturbing chemicals that can interfere with the body's own hormones. Such hormones – disturbing persistent contaminants may be hazardous at extremely low doses posing a particular threat to those exposed in the womb. During prenatal life, endocrine disturbers may alter the development and undermine the ability to learn, to fight off diseases and to reproduce.

3. CURRENT SITUATION IN EGYPT

Egypt is the second most populated country in Africa and the most populated in the Middle East, with about 72 million inhabitants. The primary activity is agriculture, representing the main component of Egypt's foreign trade. Because of the high insect pest pressure, reliance on chemical control has been well established since the early 1950s. As a result, wide use of synthetic organic pesticides has been practiced for decades for cotton, the main cash crop (El Sebaie et al., 1993).

Massive volumes of chlorinated hydrocarbons, were used in Egypt for years until their application was curtailed in the 1990s. In 1996, a Ministry of Agriculture Decree has banned the import and use of about 80 pesticides, including a large number of organochlorine pesticides such as DDT, heptachlor, chlordane, aldrin, dieldrin, chlordane, toxaphene, mirex, and endosulfan.

In recent years, Egypt has been going through relentless efforts to widen its industrial profile. Beside Egypt's well established industries such as textiles, pharmaceuticals, fertilizers, food processing, pesticides, metallurgy, plastics other industries are newly emerging such as cement, paper and pulp, petrochemicals and automobile manufacturing.

Expansion in the industrial sector resulted in an inevitable increase in the use, disposal, and release of contaminants to various segments of environmental systems.

4. ENVIRONMENTAL LEGISTLATION

One of the major repercussion of the 1992 Rio Conference on the Environment was raising environmental awareness and the emergence of new legislative bodies in several parts of the world.

In Egypt, a state ministry for environmental affairs was initially established and a new law (Law 4) for environmental protection was introduced in 1994. The new law endorsed environmental impact assessment (EIA) as a mandatory step for licensing new projects, and expansions in existing ones. EIA reports are now requiring changes in the proposed technology, or in setting certain conditions for approval. The introduction of EIA has played a pivotal role in decreasing the emission of pollutants into the environment and has had a significant effect on reducing POPs emission.

Egypt has issued a large number of laws and regulations governing the importation, manufacture, trade, and usage of chemicals covering different areas:

- The ministry of industry has used laws and regulation governing the importation, manufacture, trade and handling of chemicals used in industry.
- The ministry of agriculture has issued laws and regulations governing importation, manufacture, trade handling and usage of veterinary pharmaceutical, radioactive substances and pesticides.

A full chapter of Law 4 regulated the management of hazardous substances and wastes including chemicals. According to this law, handling of hazardous substances or wastes requires the permission of authorized agencies.

Egypt has ratified the Stockholm Convention and has accomplished its National Implementation Plan in June, 2005. However, although integrated information on POPs is limited in Egypt, the National Chemical Profile provides some information on pesticides imports and exports. Agricultural Law No. 53 issued in 1966 as well as Ministerial Decree No. 215 issued in 1985 regulated the pesticides used in Egypt. An interagency Pesticides Committee has been established under the umbrella of the Ministry of Agriculture. This committee is responsible for pesticides registration and licensing of imports. Prior to registration, a pesticide is evaluated for three successive years for its efficacy and examined at the time of registration for its chemical and physical properties.

5. EMISSION INVENTORIES

A preliminary inventory of dioxins and furans in Egypt has been prepared within the framework of the National Implementation Plan. Dioxins and furan release are estimated according to the equation:

Source Strength (dioxin emission per year)= Emission Factor × Activity Rate

Preliminary inventory of dioxin / furans release to the environment was assessed accordingly.

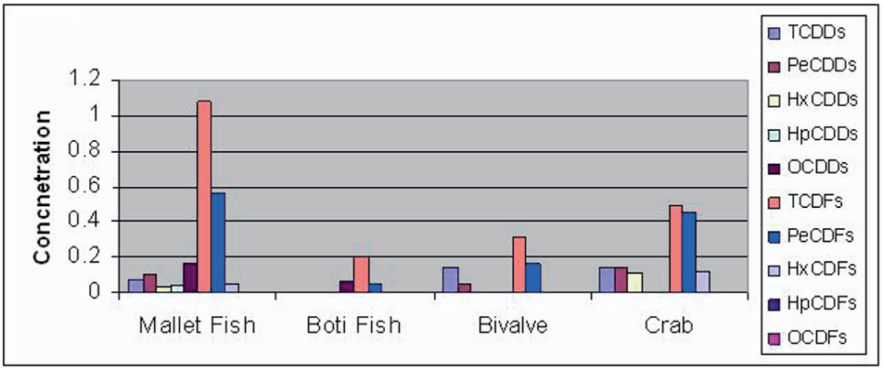

Figure 1. Residues of dioxins and furans in some marine organisms in Lake Temsah, Ismailia, Egypt.

Emission factors were produced for the following major activities:

- Municipal solid waste incineration;
- Medical waste incineration;
- Ferrous and non ferrous metal production: iron ore sintering, coke production, iron and steel production plants, copper plants, lead plants, aluminium plants, zinc production;
- Power and heat generation plants;
- Mineral products, such as cement, glass, ceramics, asphalt mixing and bricks;
- Transport;
- Uncontrolled combustion processes, waste burning and accidental fires;
- Production and use of chemicals and consumer goods, pulp and paper production, textile, chemical industries, petrochemical industries, leather refining;
- Others: tobacco smoking, dry cleaning residue, drying of biomass and others,

- Disposal/ Landfill;
- Hot spots, production sites of chlorine, chlorinated organics and PCB capacitors.

6. PERSISTENT ORGANIC POLLUTANTS MONITORING STUDIES

There is no established national monitoring scheme of POPs in the country, although the Ministries of Agriculture and Industry are responsible for the monitoring function. Several efforts were taken by regulatory bodies as well as academicians from various universities and research institutes stretching all over the country to monitor the presence of persistent organic pollutants in the environment.

7. POPS RESIDUES IN FOOD

In Egypt, emphasis is given on research on monitoring residues of POPs in food. As POPs tend to have high affinity to fats, most of these studies are focused on organochlorine pesticides and to lesser extent PCBs. The studies have focused on dairy products due to their high fat contents.

Samples of buffalo milk, powder milk and cheese were analysed for their residue content of organochlorine and cyclodiene pesticides (Dogheim et al., 1990; Tawfic Ahmed, 1991; Aman and Bluthgen, 1997; Fahmy, 1998; Amr, 1999). A common feature of these monitoring studies was the prevalence of DDT residues in all materials, but at varied levels. Buffalo milk was reported to have the highest level of ∑ DDT, at a concentration of 4240 ng/g lipid (Dogheim et al., 1990). The highest concentration level of aldrin + dieldrin, ranging between 70 to 100 ng/g lipid, was also reported in buffalo whole milk samples (Dogheim et al., 1990). However as shown in these studies an apparent decline in organochlorine residues in milk samples collected in late 1990s was observed, in comparison to those collected earlier. This trend of results is mostly related to the ban of DDT and most other cyclodiens and organochlorine pesticides in Egypt and the subsequent drop of their usage.

Malisch and Saad (1994) reported that residues of PCDD/Fs in butter samples collected from Fayoum district and Koumumbo had I – toxicity equivelant (I- TEQ) values of less than 3 pg I – TEQ/g fat, while nearly all samples collected from Lower Egypt have exceeded 5 pg I – TEQ/g fat while the mean residue of all samples was 7.69 pg I – TEQ/g fat. Residues of PCDD/Fs in meat samples collected from local markets in three different provinces had an average level of 0.94 pg TEQ/g fat (Malisch and Saad, 1994).

Residues of pesticides were determined in some randomly collected medicinal plants used for infants and adults alike. Higher level of aldrin and dieldrin residues than that set by the Egyptian Organization for Standardization and Quality Control were detected in chamomile and hibiscus tea (Abou - Arab and Abou - Donia, 2001). Similarly, Tawfic Ahmed et al. (2001a), reported a higher residue level of profenfos, an organophosphorus insecticide in some cumin samples collected from local markets around the Suez Canal provinces. Reported high residue level of profenfos could pose potential threat to children that use chamomile, considering their small body weight.

8. RESIDUES OF PERSISTENT ORGANIC POLLUTANTS IN MARINE ENVIRONMENT

Aquatic organisms are especially sensitive to contamination with persistent organic pollutants. The ability of aquatic organisms to accumulate contaminants in their tissues to elevated levels reaching concentrations that are much higher than that of ambient water, makes these biota useful for assessment purposes.

Egypt has a coastal borderline of almost 2500 km between the Mediterranean Sea, Gulf of Aqaba, Gulf of Suez and the Red Sea. A variety of land based activities influence the quality of life in Egypt's coasts, namely the Mediterranean and Red Seas. There is no report on the impact of persistent organic pollutants released from land based activities in Egypt on the quality of life of either the Mediterranean or Red Seas. However, Danovaro (2003) reported that in Egypt eutrophication has been observed in coastal waters as a result of the large nutrient input, although the Nile input was reduced by 90% in the last decades. The Department of Environment is undertaking a nationwide programme of monitoring coastal water quality with some established sampling stations stretching over the entire coastline. The monitoring programme includes salinity, coliform bacteria, dissolved oxygen, total nitrogen, nitrate, ammonia, silicate, phosphate, total phosphorus and others. Persistent organic pollutants were not included in the programme but are measured by universities and research institutes for research purposes.

9. RESIDUES OF ORGANOCHLORINE COMPOUNDS

Residues of DDTs, PCBs HCB and HCHs have been reported in fish samples collected from different locations of Egypt's Mediterranean Sea (Abd – Allah et al., 1990). DDT and its metabolites presented the highest residue levels, reaching 916 ng/g ww. Abd – Allah et al. (1998) reported that residues of DDT in mullet fish (*Mugil cephalus)* collected from different Mediterranean spots had a concentration level ranging between 4 to 9 ng/g ww., indicating a significant drop in DDT residue level that could be attributed to the reduction of organchlorine use in Egypt.

Residues of PCBs in Egypt's marine environment were reported by several researchers. Abd – Allah and Ali (1994), reported concentration levels of PCBs (Aroclor 1248), ranging between 180 and 227 ng/g ww. in fish samples collected from El Max Bay around Alexandria where many industries are located. Abd - Allah et al. (1998) reported that residues of Aroclor 1248 have ranged between 17 and 31 ng/g ww. in mullet fish *Mugil cephalus*, collected from various coastal areas, while residues detected in bivalves *Donax sp.* ranged between 19 and 46 ng/g ww.

Sediment is the main repository of pollutants in aquatic ecosystems. In a comprehensive study, Tundo et al. (2004) mapped out the concentration level of dioxin - like PCBs and other PCBs in the sediment of Lake Temsah, around Ismailia. Results indicated an elevated level in some samples with a residue concentration of 99,111 mg/kg, and an equivalent toxicity of 41,049 mg/kg. Results also showed that IUPAC No. 118 congener, 2, 3, 4, 4, 5, pentachlorobiphenyl was the most concentrated of all detected congeners, with concentrations ranging between 0.039 and 43,201 mg/kg. Di-orthocongeners (Nos. IUPAC 180 and 170) were detected in all samples alike with concentrations ranging from 0.94 to 0.55 mg/kg. The levels of PCB residues detected in this study are far lower than those detected in the sediment of Porto Marghera's lagoon (Frignani et al., 2000), sediment samples collected from south-eastern Korea (Choi et al., 2000), and from certain places in the USA (Thomas and Spiro, 1996).

Tawfic Ahmed et al. (2001b), during their study for a biological indicator to depict the conditions of Lake Temsah, Ismailia, Egypt concluded that bivalves are most suitable indicators for pollution pressure of organochlorine pesticides and PAH, in comparison to fish skin, fish gills and fish muscles. The study revealed that DDE was the most frequently detected organochlorine, while endosulfan had the highest concentration. Bivalves *Ruditapes decussata* had the widest spectrum of detected PAH, while fish skin (*Mugil cephalus*, had the least.

Residues of PCDD/Fs, dioxin like PCBs, PCBs and PAH were monitored in the sediment of Lake Temsah by Tundo et al. (2004). PCDDs and PCDFs were detected in all sediment samples collected from various sampling stations. In the PCDD group of congeners, 1, 2, 3, 4, 6, 7, 8, 9 octa-CDD had the highest concentrations in almost all samples, while 2, 3, 7, 8 tetra-CDD showed the lowest concentrations. The World Health Organization toxicity equivalents ranged from 0.387 to 11.20 ng/kg d.w. Regarding dioxin-like PCBs, results showed that IUPAC No. 118 congener 2, 3, 4, 4, 5, pentachlorobiphenyl was the most concentrated of all detected congeners, with concentrations ranging between 0.039 and 43.201 mg/kg. For polycyclic aromatic hydrocarbons, benzo(b þ k þ j)fluoranthene had the highest concentrations in almost all sampling stations. However, fluorene was found in the smallest concentration in almost all stations.

In a related study, residues of PCDDs/F, non-ortho, mono-ortho PCBs, and other PCBs were monitored in the tissues of mullet fish, bolti fish, bivalves and crab taken from Lake Temsah (Tundo et al., 2005).

Results showed that 2,3,7,8 tetra CDD and 1,2,3,7,8 penta CDD were the most frequently detected PCDD congeners while, 2,3,7,8 tetra CDF, 1,2,3,7,8 penta CDF and 2,3,4,7,8 penta CDF were the most frequently detected PCDF congeners. The mullet fish had the highest total PCDDs concentration, up to 0.398 pg/g fresh weight, followed by crab with concentration of up to 0.395 pg/g fresh weight, bivalves and bolti fish presented concentrations of about 0.187 and 0.062 pg/g fresh weight, respectively. The concentration of the mono-ortho congeners CB 118 was the highest of all non-ortho and mono-ortho PCBs congeners, with values of 0.382, 0.022, 0.231 and 0.357 ng/g fresh weight, in mullet fish, bolti fish, bivalves and crab, respectively.

Information about marine pollution by organotin and organolead are rather limited Abdel – Motie (1996) reported that alkylated lead species concentration in biota representing different levels food chain inhabiting the coastal water of Alexandria indicated higher level in demersal rather than pelagic organisms. The maximum concentration factor for crabs were 2850, 4985, 12970 and 6690 for $PbMe_3^{+,}$ $PbEt_3^{+}$, $PbMe_2^{+2}$, and $PbEt_2^{+2}$, respectively.

Reports on residues of organic pollutants in birds are rare in Egypt. Tawfic Ahmed (2005b), has mapped out organic pollutants in the vicinity of Lake Temsah,. DDE was the most prevalent pollutant, followed by dicofol and heptachlor as shown in Table 1.

Table 1. Residues of some chlorinated hydrocrbons and organophosphate compounds in some birds in Egypt μg/kg fresh weight.

Bird		DDE	Heptachlor	HCH	Dicofol	Diazanon	Malathion
Moorhen	chest	61.7	10.5	n.d.	36.5	7	34.4
	Liver	n.d.	n.d.	n.d.	12.5	23.1	116.7
Coot	chest	56.0	1.5	n.d.	n.d.	n.d.	n.d.
	Liver	4	n.d.	n.d.	n.d.	n.d	49.7
Teal	chest	n.d.	n.d.	4	7.32	n.d	n.d.
	Liver	n.d.	n.d.	n.d.	n.d.	36	n.d.
Cormorant	chest	n.d.	n.d.	n.d.	n.d.	n.d	n.d.
	Liver	11.3	n.d.	n.d.	n.d.	14.3	148.8
Black headed gull	chest	11.6	19.43	2.3	15	n.d.	n.d.
	Liver	1.2	0.8	n.d.	0.8	79.6	256.7
P. Snipe	chest	0.4	n.d.	n.d.	n.d.	n.d.	n.d.
	Liver	n.d.	n.d.	n.d.	n.d.	36.7	n.d.
Crane	Chest	0.18	n.d.	n.d.	n.d.	n.d.	n.d.
	liver	n.d	n.d.	n.d.	n.d.	n.d.	45

10. RESIDUES IN HUMAN

Studies on the residues of persistent organic pollutants in man are focused on human milk. Dogheim et al. (1988, 1991, 1996) have mapped out residues of organochlorine pesticides in samples collected from different parts of the country. DDTs were the most frequently detected compound in Cairo area with the concentration varying between 4 and 257 ng/g fresh weight. Similar trend of results were reported by El Shiekh et al. (1990) on human milk samples collected from rural areas around Ismailia. Saleh et al. (1996), examining human milk samples collected from some local governotates, concluded that the mean concentration of p,p-DDE and p,p-DDT were in the range of 21 and 2.9 ng/g on a whole milk basis. The study has also revealed that WHO recommended limits (1986) were not exceeded in all samples. In another study dealing with DDT residues in samples collected from a wider community, it was revealed that samples exceeding the acceptable daily intake for children (ADIs) set jointly by FAO/ WHO was in the range of 7 to 48% (Saleh et al., 1996).

Tawfic Ahmed (1995) studied the influence of dietary habits on residues of organochlorine pesticides in human urine. Urine samples collected from fishermen around Lake Manzala, Port Said, with a diet based mainly on polluted fish caught from the lake, were compared to urine samples of persons living in the city with more diversified food. Results showed that urine samples collected from fishermen had a higher frequency, a wider spectrum, and higher concentrations of organochlorine pesticides. DDT, DDD, DDE and dieldrin residues in fishermen urine were 68, 39, 97, and 53 ng/L respectively. In the other group of people with diversified food, only residues of DDT and DDE were detected at concentrations of 27 and 38 ng/L respectively, while DDD and dieldrin were not detected.

Tawfic Ahmed et al. (2002) studied residues of certain persistent organic pollutants is blood samples collected from several groups of women to ascertain the relationship between body burden of persistent organic pollutants and breast cancer. The study was conducted to detect residues of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and polychlorinated biphenyls (PCBs) in blood serum samples collected from a cohort of fasting females attending the health insurance outpatient clinic at Port Said. Females involved in the study included those diagnosed with invasive adeno-carcinoma of the breast, females suffering benign breast disease, and normal healthy females. Mean residues of DDE detected in the three examined groups of females were 41±5.2, 48±6.2 and 31±2.5 ng/g for breast cancer cases, benign breast disease cases and controls, respectively, indicating some significantly lower residues in blood serum of control females.

11. FRESHWATER ECOSYSTEMS

Water resources in Egypt are rather limited and the Nile River provides almost 95% of the country’s demands. Because of the extensive agricultural activities around the Nile valley and its delta, massive volumes of pesticides have been used. Water bodies have been subjected to various levels of pesticides contamination especially organochlorine pesticides. Several studies have reported high levels of pesticides contamination in water bodies, but mainly focused on organochlorine pesticides. Varied levels of DDTs, isomers of HCH, and other cyclodiens were monitored in different segments of fresh water ecosystems (Dogheim et al., 1988, 1990, 1996; Yamashita et al., 2000). On the other hand, reports on POPs other than organochlorines in freshwater are very limited. Tawfic Ahmed and Ismail (1999) reported the concentration of PAH in rain and groundwater in the vicinity of Ismaila. Residue levels of 7 PAHs were monitored in rain samples collected in two successive years, 1995 and 1996. Total PAH residues were 26 and 30 μg/L while the total residue of PAHs in groundwater was in the range of 8.0 μg/L, with anthracene constituting the major PAH compound. In a similar study, residues of DDT along with some cyclodiene pesticides were detected in rain water collected during two successive years. A total residue load of 4.7 and 10.6 μg/L was detected in 1995 and 1996, respectively (Tawfic Ahmed et al., 1998).

12. GREEN CHEMISTRY

The impact of POPs on human and environmental health has been of serious tragic nature. Incidents of the sort of Agent Orange that hit the Vietnamese and Sevso, Italy are deeply embedded in human consciousness as milestones of the unleashed power of chemistry when it goes wrong. The European Chemical Industry Council (ECIC) survey in 1994 showed that 60% of the general public had an unfavourable view of the chemical industry and in the USA, a survey carried out for the Chemical Manufacturers Association (CMA) in 1993 showed that only 26% were favourably disposed towards the industry. Chemistry has been seen by people at large as the most potential risks to sustainability and to a sustainable future. As Clark (1999) put it "chemical industry and chemists are actually seen by many as causes of the problems".

The closing decades of the last century have also witnessed the emergence of green chemistry, thanks to the newly conceptualized ideas of renowned chemists such as Barry Trost and others. Green Chemistry is defined as the prevention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances. In chemical synthesis, the ideal will be a combination of a number of environmental, health and safety considerations along with economic targets. A number of international and regional organizations have emerged in USA, Italy and UK with green chemistry as the core theme. At the Mediterranean level, a newly established network for green chemistry (MEGREC) has recently been set up. Under this new network, application of green chemistry is most likely to make a positive impact in the Mediterranean countries if incorporated in selected development programs targeting key areas such as the regulation that govern the use and disposal of POPs. Egypt is one of founders of the network with zealous efforts to improve the quality of life through improving practices and use of chemicals. Green chemistry is one of the courses currently introduced in the curricula of some Egyptian universities.

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Fate and Behavior of Anthropogenic Pollutants in Soils and Water

2.1. Fundamental Issues in Sorption Related to Physical and Biological Remediation of Soils
Joseph J. Pignatello

2.2. The role of humic substances in the fate of anthropogenic organic pollutants in soil with emphasis on endocrine disruptor compounds
Elisabetta Loffredo and Nicola Senesi

2.3. Incorporating bioavailability into criteria for metals
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Barbara Jezierska and Małgorzata Witeska

FUNDAMENTAL ISSUES IN SORPTION RELATED TO PHYSICAL AND BIOLOGICAL REMEDIATION OF SOILS

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Abstract: Sorption of organic contaminants to natural particles plays a fundamental role in their biological availability. This paper considers the influence of sorption thermodynamics and kinetics on contaminant bioavailability, with emphasis on the author's prior work. Equilibrium bioavailability is evaluated by examining the effects of sorption behavior on the Biomembrane-Soil Organic Carbon Concentration Factor ($BS_{OC}CF$), defined as the ratio of biomembrane-water to soil OC-water distribution coefficients. Phosphatidylcholine liposomes are used to model the biomembrane phase. The influence of sorption kinetics is discussed for circumstances in which desorption from particles is rate limiting to bio-uptake. Factors examined include the influence of contaminant structure, contaminant concentration, the concentration of competing co-solutes, and irreversible sorption phenomena such as hysteresis and conditioning effects. The generation of a highly desorption-resistant fraction and the underlying causes of desorption resistance are discussed, especially in the context of a 'matrix collapse' outcome of irreversible sorption. A section is included on 'facilitated bioavailability,' in which cells may access sorbed molecules directly or are able to–promote desorption by altering the chemical or physical properties of the soil particle or surrounding liquid.

Key words: liposomes; dual mode model; polycyclic aromatic hydrocarbon; polychlorinated biphenyl; bioremediation; glassy polymer; nonlinear sorption; competitive sorption; sequestration; pore deformation hypothesis

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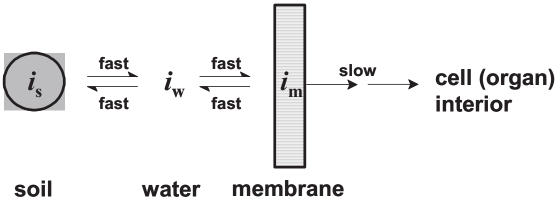
1. INTRODUCTION

Sorption plays a role in all aspects of contamination of soils and sediments by organic compounds. Sorption governs the transport of contaminant molecules, regulates the fluid-phase concentration surrounding the biological membrane through which contaminant molecules must pass to exert a toxic effect, and governs the availability of contaminant residues for physical-chemical or biological removal. The study of sorption has been a major branch of environmental science for decades because of its central importance. Usually, the emphasis is on the role of sorption in contaminant transport in the environment. This paper will focus primarily on the influence of sorption on bioavailability. It will mainly review the work of our group directed toward an understanding of the thermodynamics and kinetics of sorption regarding its influence on bioavailability.

2. THERMODYNAMIC REGULATION OF BIOAVAILABILITY

The uptake of a pollutant molecule by an organism occurs after that molecule has passed from the gas or aqueous solution phase through a biological membrane (cell membrane or higher-order membrane) and into the interior of the organism where it can accumulate, be metabolized, or exert a toxic effect. There is no convincing evidence that molecules can pass directly from the soil-sorbed state to the membrane, bypassing the fluid phase. The uptake of most anthropogenic organic pollutants is believed to be passive — that is, it occurs by diffusion through the membrane rather than by active transport processes. The flux into the cell at any instant is governed by the concentration existing at the interior interface of the biomembrane.

To evaluate the thermodynamic regulation of bioavailability by sorption for a contaminant, *i*, we consider the situation in which the sorbed, dissolved, and the membrane-bound states are close to equilibrium, and in which the rate-limiting step is mass transport from the membrane into the interior~~,~~ or some other intracellular step (Scheme 1).



(Scheme 1)

This situation is realistic when sorption and membrane partitioning are rapid compared to intracellular steps~~,~~ or if the receptor does not appreciably reduce the chemical concentration immediately surrounding it during the duration of exposure. Partitioning of contaminant into the vapor phase in Scheme 1 is omitted for simplicity. Vapor-phase concentration is related to solution-phase concentration by the Henry's law coefficient.

It is widely accepted that sorption of most neutral organic compounds occurs predominantly to the soil organic matter (SOM) fraction. The model historically (and still most frequently) used to describe sorption to SOM is in the linear partition model:

$$C_\mathrm{s} / f_\mathrm{oc} = C_\mathrm{oc} = K_\mathrm{ocw} C_\mathrm{w} \tag{1}$$

where C_s [mol/kg$_\mathrm{s}$] is the sorbed concentration in the whole soil; f_oc is the fraction of soil organic carbon (OC); C_oc is the OC-based sorbed concentration [mol/kg$_\mathrm{oc}$], C_w is the aqueous solute concentration [mol/L$_\mathrm{w}$] , and K_ocw is OC-water partition coefficient [L$_\mathrm{w}$/kg$_\mathrm{oc}$].

Likewise, the equilibrium expression for partitioning between water and the biomembrane is given by:

$$C_\mathrm{m} = K_\mathrm{mw} C_\mathrm{w} \tag{2}$$

where C_m is the membrane-phase concentration [mol/kg$_\mathrm{m}$ or mol/L$_\mathrm{m}$] and K_mw is the membrane-water partition coefficient [L$_\mathrm{w}$/kg$_\mathrm{m}$ or L$_\mathrm{w}$/L$_\mathrm{m}$].

We may now define the Biomembrane-Soil Organic Carbon Concentration Factor (BS$_\mathrm{oc}$CF) [kg$_\mathrm{oc}$/kg$_\mathrm{m}$] as the ratio of concentrations in the membrane and the soil, obtained by combining eqs 1 and 2:

$$\mathrm{BS_{oc}CF} = \frac{C_\mathrm{m}}{C_\mathrm{oc}} = \frac{K_\mathrm{mw}}{K_\mathrm{ocw}} \tag{3}$$

We will see in subsequent discussion how the BS$_\mathrm{oc}$CF is affected by various factors influencing the sorption process.

2.1 Effect of Contaminant Structure

Extensive literature is available on the effect of solute structure on sorption by soils from aqueous solution. The most useful correlation is the linear free energy relationship (LFER) between OC sorption and 1-octanol-water partitioning:

$$\log K_{ocw} = a_{oc} \cdot \log K_{ow} + b_{oc} \quad (4)$$

where K_{ow} is the 1-octanol-water partition coefficient and a_{oc} and b_{oc} are regression coefficients. This correlation is strong for a related set of compounds. A linear relationship with a similar slope may also be constructed between log K_{ocw} and the solute molar volume. The K_{ow} and the solute molar volume primarily reflect ‘hydrophobic effects,’ which have to do with expulsion of organic molecules from the less favorable environment of the aqueous phase into a more favorable environment of the organic phase. When the dataset includes compounds that are not closely related—especially when it includes a mix of polar and apolar compounds—it is more accurate to use multi-parameter LFERs that take into account the net effects of all possible weak interactions (London, Keesom, Debye, H-bonding, etc.) that occur between the molecule and its surroundings in the sorbed and dissolved states (Goss and Schwarzenbach, 2001; Nguyen et al., 1995). However, for our purposes the simpler single-parameter model is satisfactory.

Values of K_{ocw} have been determined for many compounds in many different soils and sediments. Regression curves for “polycyclic aromatic hydrocarbons (PAHs),” “halogenated hydrocarbons,” and “polar compounds” collected from the literature by Nguyen et al. (2005) are shown in Figure 1.

A LFER may likewise be constructed between biomembrane-water partitioning and 1-octanol-water partitioning:

$$\log K_{mw} = a_{m} \cdot \log K_{ow} + b_{m} \quad (5)$$

where a_m and b_m are regression coefficients. Chemical partitioning in real biomembranes is experimentally difficult and instead researchers have used models. The simplest and most widely accepted models are artificial bilayer phospholipid vesicles based on phosphatidylcholine monomers known as *liposomes*. Partitioning of nonionic compounds (or the neutral form of ionogenic compounds) in liposomes is characteristically linear with concentration, except at aqueous concentrations approaching solubility (Escher and Schwarzenbach, 1996). Figure 1 includes the regression curves for selected liposome systems. The log K_m-log K_{oc} relationship is essentially linear for a closely-related set of chemicals up to about log K_{ow} ~7. Above log Kow ~7, the slope of the relationship for polychlorinated biphenyl (PCB) partitioning in liposomes seems to decline, and even become slightly negative. This has been attributed to steric effects in packing of the highly chlorinated congeners (Dulfer and Govers, 1995).

Combining eqs 4 and 5 with eq 3 gives an expression for the $BS_{oc}CF$ as a function of K_{ow}:

$$\log(BS_{oc}CF) = (a_m - a_{oc}) \cdot \log K_{ow} + (b_m - b_{oc}) \quad (6)$$

Figure 2 plots eq 6 for selected combinations of data sets represented in Figure 1. Similar plots can be made by substituting solute molar volume for log K_{ow}. From the graph in Figure 2 it can be seen, first, that there is little dependence of $BS_{oc}CF$ on K_{ow} below log K_{ow} ~7. This means that among neutral compounds of the same type in a given soil, the equilibrium bioavailability is almost independent of hydrophobic character or molecular

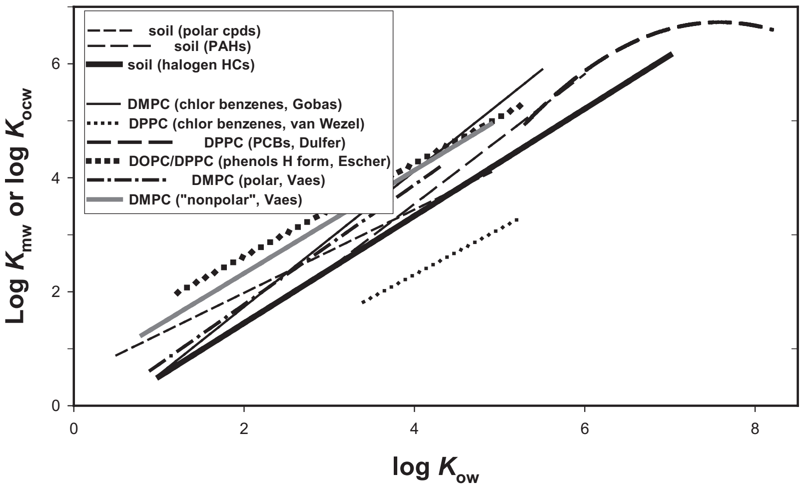


Figure 1. Relationship liposome-water and soil organic carbon-water partition coefficients to n-octanol-water partition coefficient. DOPC = L-α-dioleylphosphatidylcholine; DPPC = L-α-dipalmitoylphosphatidylcholine; DMPC = dimyristoylphosphatidylcholine. The correlations for 33 halogenated hydrocarbons in soil ($\log K_{ocw} = 0.94 \log K_{ow} - 0.43$), 11 PAHs in soil ($\log K_{ocw} = 1.14 \log K_{ow} - 1.02$), and 21 polar compounds in soil ($\log K_{ocw} = 0.73 \log K_{ow} - 0.52$) are from Nguyen et al. (2005). The correlation for chlorinated benzenes in DMPC ($\log K_{mw} = 1.19 \log K_{ow} - 0.645$) is from Gobas et al. (1988), for seven chlorinated benzenes in DPPC at 23 °C ($\log K_{mw} = 0.80 \log K_{ow} - 0.90$) is from van Wezel et al. (1996), for fourteen PCBs in DPPC ($\log K_{mw} = -0.343 (\log K_{ow})^2 + 5.20 \log K_{ow} - 12.98$) is from Dulfer and Govers (1995), for polar compounds in DMPC ($\log K_{mw} = 0.904 \log K_{ow} + 0.515$) is from Vaes et al. (1997), and for nonpolar compounds in DMPC ($\log K_{mw} = 1.05 \log K_{ow} - 0.324$) is from Vaes et al. (1997). The correlation for twenty neutral phenols in DOPC/DPPC (8:2 ratio) is the regression line ($\log K_{mw} = 0.815 \log K_{ow} - 0.989$; $r^2 = 0.958$) through data tabulated in Escher and Schwarzenbach (1996).

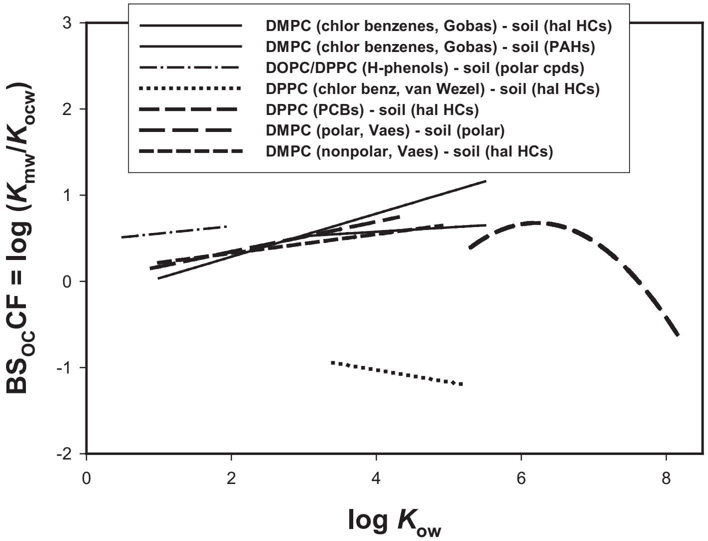


Figure 2. Relationship between Biomembrane-Soil Organic Carbon Concentration Factor and *n*-octanol-water partition coefficient for different series of compounds represented in Figure 1. For the PCB curve, the K_{oc}-K_{ow} LFER was extended a log unit beyond the included data set to log $K_{ow} = 8$.

size. For example, benzene and the four-ring PAH, pyrene are predicted to have nearly the same bioavailability under equilibrium conditions. Second, there is less than about a factor-of-three difference between polar and apolar compounds. Third, log $BS_{oc}CF$ ranges between 0 and 1 in most cases, which means that the distribution tends to modestly favor the membrane phase over the soil OC phase as a whole. An exception is the data set based on chlorinated benzene partition coefficients in DPPC (van Wezel et al., 1996) that is in substantial disagreement with the others.

2.2 Effect of Contaminant Concentration

Equation 3 assumes that sorption in both the membrane and soil is concentration-independent. While usually the case for liposomes, it is not generally the case for soils. An isotherm model that has been widely employed is the Freundlich model:

$$C_s / f_{oc} = C_{oc} = K_{F,ocw} C_w{}^n \tag{7}$$

where $K_{F,ocw}$ is the OC-based Freundlich coefficient and n is the Freundlich exponent, which can take on any value, but is usually less than 1. A value less than 1 indicates decreasing affinity for the solid with increasing concentration. The Freundlich model does not always fit the data perfectly, but it is useful for our purposes. The expression for $BS_{oc}CF$ incorporating nonlinearity of soil sorption is:

$$BS_{oc}CF = \frac{C_m}{C_{oc}} = \frac{K_{mw}}{K_{F,ocw}} C_w^{\,1-n} \tag{8a}$$

or

$$BS_{oc}CF = \frac{C_m}{C_{oc}} = \frac{K_{mw}}{K_{F,ocw}^{\,1/n}} C_{oc}^{\,\frac{1-n}{n}} \tag{8b}$$

depending on whether $BS_{oc}CF$ is expressed as a function of solute or sorbate concentration.

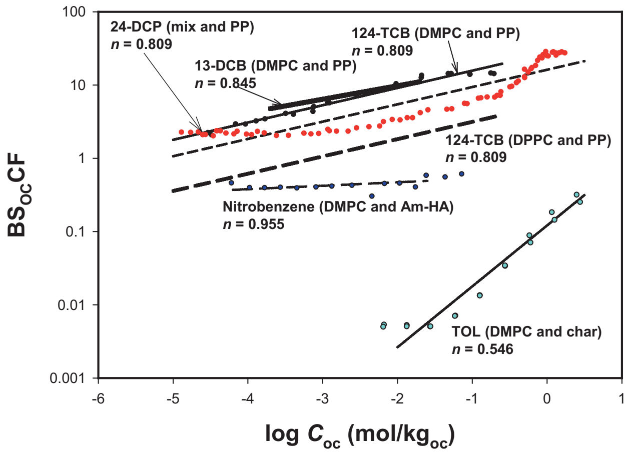


Figure 3. Biomembrane-Soil Organic Carbon Concentration Factor ($BS_{oc}CF$) for selected compounds. Given is n, the Freundlich exponent. 1,3-Dichlorobenzene and 1,2,4-trichlorobenzene in DMPC and DPPC liposomes used measured K_{mw} values listed in van Wezel et al. (1996). 2,4-Dichlorophenol used measured K_{mw} value from Escher and Schwarzenbach (1996). Toluene and nitrobenzene K_{mw} values were calculated by the liposome-octanol LFER expression of Gobas et al. (1988).

Figure 3 plots $BS_{oc}CF$ versus sorbate concentration for selected systems where the soil isotherms are known in detail and when the liposome-water partition coefficients have been measured or can be estimated by the K_m-K_{ow} LFERs in Figure 1. The examples displayed include both polar and apolar compounds and isotherms that vary from almost linear to highly nonlinear. Shown are actual data calculated as C_m/C_{oc} as well as the Freundlich model predicted curves. The $BS_{oc}CF$ is nearly constant for compounds whose isotherms are close to linear (e.g., nitrobenzene in a humic acid ($n = 0.955$). By contrast, compounds that give strongly nonlinear isotherms in soil have $BS_{oc}CF$ values that are correspondingly non-constant with concentration. By example we may consider partitioning of toluene between a liposome and charcoal, which is a form of "black carbon" present in many soils and believed to be an especially strong adsorbent of organic compounds. The isotherm of toluene on charcoal is highly nonlinear ($n = 0.546$); accordingly, the $BS_{oc}CF$ increases by two orders of magnitude over the experimental concentration range of slightly more than two orders of magnitude. Compounds with more typical Freundlich n values, such as 2,4-dichlorophenol and 1,2,4-trichlorobenzene in a peat soil ($n = 0.809$ in both cases) give intermediate results. In these cases, $BS_{oc}CF$ increases by about one order of magnitude over about five orders of magnitude in concentration.

One can expect that as concentration falls during the course of degradation, uptake or through dilution of the liquid phase, the membrane becomes less and less effective from a thermodynamic standpoint in attracting contaminant molecules. Molecules sorbed to black carbon are less bioavailable than molecules sorbed to SOC as a whole, and become even less so as uptake or degradation proceeds.

2.3 Competition for Sorption by Co-Solutes

When a nonlinearly sorbing compound is present in a system containing one or more additional compounds there may be competition for mutually accessible "sites." This follows from the fact that nonlinear sorption is a form of self-competition. Competitive effects are important because contaminant mixtures occur far more commonly than single contaminants. Competitive equilibrium sorption seems to be fairly commonly observed in soil and sediment systems containing two or three organic solutes and can be predicted using established competitive sorption models (Pignatello, 1991; Xing et al., 1996; Xing and Pignatello, 1997, 1998; White et al., 1999; White and Pignatello, 1999; Zhao et al., 2001, 2002). In addition, competitive effects have been observed between contaminants and naturally-occurring aromatic acids (Xing et al., 1998). Competitive sorption in liposomes or real biomembranes has not been addressed experimentally, but is expected to be absent for at least two reasons. First, the fluid-like nature of the membrane is conducive to sorption linearity, except possibly at concentrations approaching solubility. Second, active membrane transport, which could involve association at specific sites on the membrane, is widely believed unlikely for most pollutants.

An example of the potential effect of competitive sorption on equilibrium bioavailability is illustrated here. Zhao et al. (2002) reported the suppression of anthracene sorption in estuarine sediment in the presence of phenanthrene and pyrene. The isotherm of anthracene was displaced downward and became more linear in the presence of the other two solutes. Figure 4 shows that the $BS_{oc}CF$ of anthracene calculated for a DMPC-estuarine sediment system increases by an order of magnitude or more in the presence of the other two PAHs. The dependence of $BS_{oc}CF$ on phenanthrene concentration in the tri-solute system is almost eliminated, consistent with an increase in the linearity of its isotherm when the other PAHs are present.

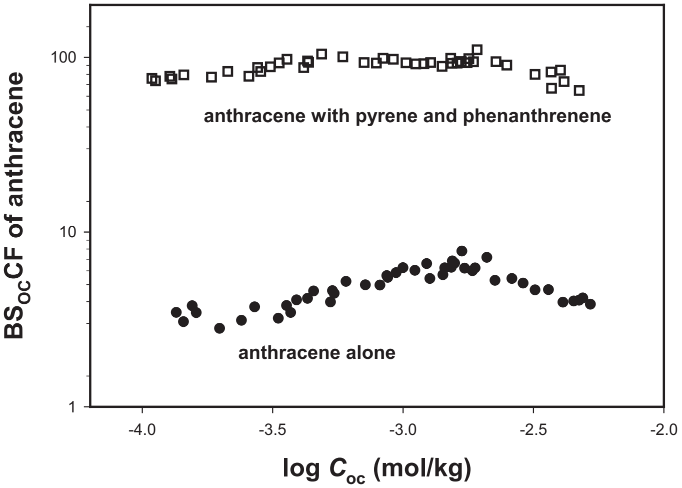


Figure 4. Effect of co-solute competitive sorption on Biomembrane-Soil Organic Carbon Concentration Factor calculated for anthracene. Model membrane: DMPC liposomes; soil: estuarine sediment from Long Island Sound (Zhao et al., 2002). Competing solutes: phenanthrene and pyrene. The K_m for anthracene in DMPC was calculated using the K_m-K_{ow} LFER of Gobas et al. (1988).

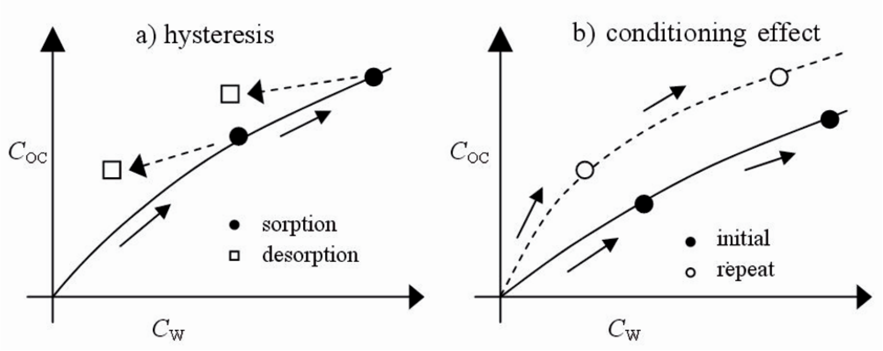
There is also direct evidence that bioavailability is enhanced when a competitor is present. Mineralization of phenanthrene in two different soils by *Pseudomonas* Strain R, a culture grown up on phenanthrene, was enhanced when pyrene was added (White et al., 1999). Pyrene was non-biodegradable by this organism and probably did not induce the degradation of phenanthrene. In parallel sterile experiments, it was shown that pyrene added after equilibration of phenanthrene quickly displaced phenanthrene into solution, resulting in a reduction in the apparent soil-water distribution ratio of phenanthrene by up to 44 to 83% relative to a control (White and Pignatello, 1999).

A reduction in the distribution ratio of a principal solute by a competing co-solute results in an increase in the aqueous concentration of the principal solute, which, in turn, leads to a higher concentration in the biomembrane phase, and therefore enhanced bioavailability of the principal solute. One may anticipate that if the co-solute is consumed by organisms, or otherwise lost from the system, along with the principal solute, the bioavailability of the principal solute would decline accordingly.

2.4 Effect of Irreversible Sorption Behavior

Physi-sorption of organic compounds is commonly assumed to be reversible. It is becoming clear, however, that this assumption is not generally valid: sorption depends on the prior sorption history of the sample. This can be manifested in two ways: by *hysteresis* in the isotherm, i.e., the non-singularity of the sorption and desorption branches (Scheme 2a), or by the *conditioning effect*, which refers to the enhanced sorption observed in a repeated experiment on the same sample after a prior sorption-desorption cycle (Scheme 2b). When not due to artificial causes, these phenomena comprise *irreversible sorption*. The term irreversible is used in the thermodynamic sense to mean that the pathways for desorption and sorption are different. It does not necessarily imply irretrievability of the chemical. In both hysteresis and conditioning phenomena one can see that sorption is enhanced in a subsequent experiment, whether it be desorption or repeat sorption.

Hysteresis is observed after diluting the fluid phase of an equilibrated system to induce desorption and finding that the newly-equilibrated point does not fall back on the original sorption isotherm. While artificial causes of hysteresis are possible, Sander and Pignatello (2005b) have verified true hysteresis in several cases using isotope exchange techniques. In systems exhibiting hysteresis, they found complete exchange when a tiny amount of ^{14}C-labelled chemical was added or removed at the initial bulk-chemical



Scheme 2

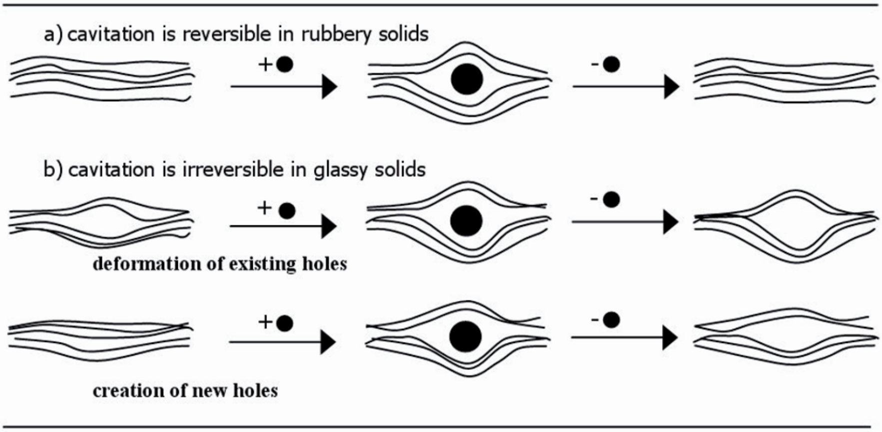
sorption or desorption points, keeping bulk chemical concentration constant. This indicates that sorption is reversible for infinitesmal changes in concentration, but is irreversible (i.e., truly hysteretic) for large changes in concentration.

The conditioning effect is observed by constructing a sorption isotherm on a sample that previously sorbed the chemical of interest or some other chemical serving as a "conditioning agent." (The conditioning agent is removed before the isotherm is constructed.) The conditioning effect is revealed by comparing the isotherm on the conditioned sample with that on a control sample that had no previous history of sorption, but was carried though the same physical manipulations (Xia and Pignatello, 2001; Lu and Pignatello, 2002, 2004; Sander et al., 2006).

It is not possible thermodynamically for a given solute concentration to correspond to multiple sorbed concentrations. It follows that irreversible sorption must arise from the generation of metastable states. The cause of hysteresis in sorption of organic compounds to natural organic matter has been attributed (Lu and Pignatello, 2002, 2004; Sander et al., 2005; Sander et al., 2006) to the same mechanism that generally causes hysteresis in glassy polymers and macromolecular organic solids. (The polymer analogy for natural organic matter is well accepted among environmental scientists.) This mechanism for hysteresis is referred to as "pore deformation."

Non-crystalline organic solids such as polymers may exist in the rubbery (flexible-chain) state or the glassy (stiff-chain) state. Which state the solid assumes depends on its chemical composition, temperature, and the concentration of plasticizers. Plasticizers are chemical absorbents — including the absorbing chemical of interest — that soften the polymer and increase the chain flexibility by virtue of forming plasticizer-chain interactions at the expense of chain-chain interactions. For example, phthalate esters are often added to stiff plastics to make them pliable.

The glassy state of an organic solid is perpetually metastable because the stiffness of its chains prohibits its complete relaxation to the thermodynamic state. As a result, the glassy state contains excess free—volume—essentially packing defects in the form of molecular-scale cavities, or "holes." In order to understand irreversible sorption in the context of the pore deformation mechanism one must realize that for absorption to occur in either rubbery or glassy solids, a cavity of appropriate size must pre-exist or be created inside the solid to accommodate the incoming sorbate molecule, as illustrated in Scheme 3.



(Scheme 3)

In rubbery solids, cavitation is more-or-less reversible during a sorption-desorption cycle because the chains are sufficiently flexible and can relax on a timescale that is as short or shorter that the timescale of desorption. For glassy solids, however, cavitation is not always irreversible due to the inflexibility of the macromolecules. Molecules entering pre-existing holes that are too small may induce expansion of the hole that then does not fully relax when the molecule leaves; likewise, molecules entering areas lacking excess free volume may create a cavity that does not fully relax when the molecule leaves. In both cases, the solid is left with a higher free volume after desorption. Accordingly, this results in a higher affinity for sorbing molecules in a subsequent sorption experiment because the free energy of hole expansion/creation has already been invested by the previous sorption step. Annealing the conditioned soil or humic acid samples at temperatures up to 90°C leads to a time- and temperature-dependent relaxation of the sorption distribution coefficient towards original values (Lu and Pignatello, 2002; Sander et al., 2006), indicating that matrix relaxation is activated, and providing convincing support for the pore deformation mechanism.

Figures 5 and 6 illustrate how sorption irreversibility can affect bioavailability. Figure 5a shows the hysteretic isotherm of 1,4-dichlorobenzene in a soil, which is one of several isotherms in various soils that has been identified as being truly hysteretic after rigorous experimentation to rule out artifacts (Sander and Pignatello, 2005b). Hysteresis in this example is considered to be moderate; that is, the Thermodynamic Index of Irreversibility (TII) is calculated to be 0.3-0.6, depending on concentration, on a scale of 0 to 1 (Sander et al., 2005). Figure 5b plots the $BS_{oc}CF$ for sorption and desorption data in Panel a and shows that hysteresis is expected to lead to a decrease of up to about a factor of two in thermodynamic bioavailability.

Figure 6a shows the isotherms of 1,2,4-trichlorobenzene in a soil before and after conditioning with chlorobenzene (Sander et al., 2006) one sees that the conditioning effect in these examples leads to up to a factor of three increase in sorbed concentration at a given solute concentration after conditioning. Similar results were obtained for 1,3-dichlorobenzene in a soil humic acid (Sander et al., 2006), for trichloromethane in a soil (Xia and Pignatello, 2001), and for chlorinated benzenes in glassy poly(vinyl chloride) (Sander et al., 2006). (As expected, the conditioning effect was not found for rubbery polyethylene (Sander et al., 2006).) Figure 6b shows that conditioning leads to a reduction of the $BS_{oc}CF$ for 1,2,4-trichlorobenzene in Pahokee peat by a factor of about two in the conditioned sample compared to the control.

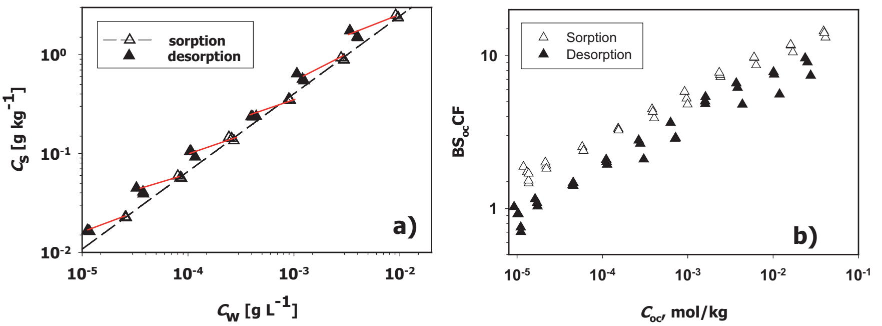


Figure 5. Effect of sorption irreversibility manifested as hysteresis on the calculated biomembrane-soil organic carbon concentration factor ($BS_{oc}CF$) for 1,4-dichlorobenzene on Pahokee peat. (a) sorption and single-step desorption points; dashed line is fit of sorption data to the Freundlich equation and solid lines link desorption replicate points to their corresponding origin on the sorption branch. (b) $BS_{oc}CF$ versus solid-phase concentration for the system in panel a, using experimental data for DMPC liposomes (Gobas et al., 1988).

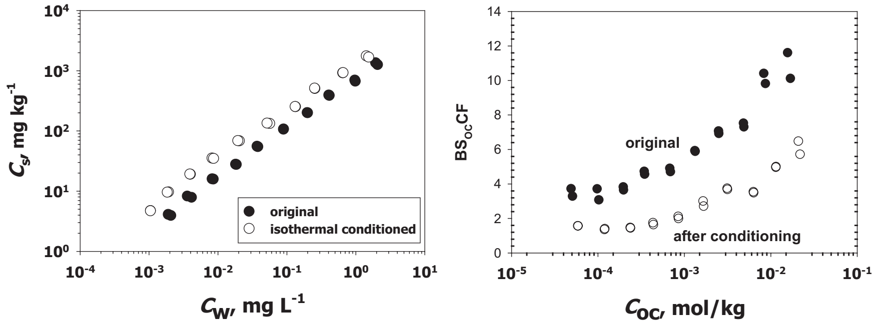
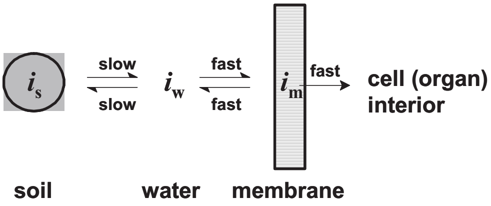


Figure 6. Effect of sorption irreversibility (manifested as the conditioning effect) on the calculated biomembrane-soil organic carbon concentration factor (BS$_{oc}$CF) for 1,2,4-trichlorobenzene on Pahokee peat soil. (a) sorption isotherms (Sander et al., in press). (b) BS$_{oc}$CF with respect to DMPC liposomes corresponding to Panel a.

3. MASS TRANSPORT LIMITATIONS TO BIOAVAILABILITY

When nonequilibrium conditions prevail during exposure it is necessary to consider mass transport rate laws describing the flux of chemical through the particle, across the particle-bulk fluid interface, across the fluid-biomembrane interface, and through the biomembrane phase. These fluxes are dictated by convection and/or diffusion rate laws applicable to each phase, which, in turn, depend on the geometry of the phase, the concentration gradient that exists within the phase at any given time, and the boundary conditions. All-encompassing rate laws are not easy to write given the heterogeneity of the system. Although few data are available, it may be predicted that intra-membrane mass transport is relatively rapid in view of the fluid nature of the membrane and its thickness. Experience indicates that intra-particle mass transport, by contrast, may be relatively slow; depending on the compound and soil, equilibrium in a spiked aqueous soil suspension may require as short as a few hours or as long as several months (Pignatello and Xing, 1996). It will often be the case, then, that that incorporation of a contaminant by an organism will eventually become rate-limited by intra-particle mass transport. This is illustrated in Scheme 4.



(Scheme 4)

3.1 Effect of Solute Structure

The most systematic studies in this regard were performed by Brusseau and co-workers using frontal analysis of elution profiles obtained in soil column experiments. The analysis was based on the "two-site" model, which postulates instantly-reversible and slowly-reversible compartments. While this distinction is artificial, the results can be used for comparative purposes. The log of the rate parameter for the slowly-reversible compartment for a given soil correlated inversely with the solute's equilibrium sorption affinity, such as log soil-water partition coefficient (Borglin et al., 1996), the first-order valence molecular connectivity index—a measure of molecular topology—(Piatt and Brusseau, 1998), or log K_{ow} (Piatt and Brusseau, 1998). Other studies with more limited data sets confirm these trends, at least qualitatively. Such behavior is consistent with the notion that the stronger the compound sorbs, the slower will be its progress of random migration through intraparticle pores or the organic matter matrix to/from the bulk water interface.

3.2 Effect of Solute Concentration

It is predicted theoretically that normalized sorption or desorption rate or the calculated diffusion rate parameter will be concentration-dependent for compounds showing a nonlinear isotherm, and concentration-independent for compounds showing a linear isotherm (Braida et al., 2001, 2002; Zhao et al., 2001, 2004). For example, for a compound with Freundlich $n < 1$, sorption becomes progressively weaker as concentration increases; thus, normalized rate or diffusion parameter is expected to increase with final concentration in the uptake direction or with starting concentration in the release direction.

In many cases we have found that the dual-mode model (DMM), originally proposed in connection with isotherms of gases and organic vapors in glassy polymers, fits sorption isotherms of chemicals in soil better than the Freundlich model (Xing and Pignatello, 1997; Xia and Pignatello, 2001). The DMM, which applies to natural organic matter as the sorbent, contains a dissolution domain (described by a linear term) and a hole-filling domain (described by a Langmuir term):

$$C_{\mathrm{OC}} = K_{\mathrm{D}} C_{\mathrm{W}} + \frac{C_{\mathrm{OC}}^{\max} \cdot b \cdot C_{\mathrm{W}}}{1 + b \cdot C_{\mathrm{W}}} \tag{9}$$

where K_{D} is the dissolution-domain partition coefficient and $C_{\mathrm{OC}}^{\max}$ and b are the hole-filling domain capacity and affinity coefficients, respectively. If one assumes—as in the polymer model—that molecular "jumps" between holes and the surrounding dissolution domain are much slower than jumps within the dissolution domain, the apparent diffusion coefficient (D_{app}) can be written as the product of a concentration-independent diffusion coefficient reflecting diffusion within the dissolution domain and a concentration-dependent term reflecting exchange between dissolution and hole sites (Zhao et al., 2002). Thus, D_{app} is expected to be independent of concentration at very low and very high concentrations, where as inspection of equation 9 reveals the isotherm is linear, and dependent on concentration at intermediate levels, where the isotherm is nonlinear.

The expectation of concentration-dependent kinetics is met experimentally, with some complications due to shifting boundary conditions (Braida et al., 2001, 2002; Zhao et al., 2001, 2004). In all these studies sorption or desorption is found to increase in rate with increasing final or initial concentration, respectively. Recently, isotope exchange experiments, in which ^{14}C-labelled chemical exchange rates were followed after equilibration of bulk chemical at two widely-spaced concentrations, clearly showed that sorption and desorption are both relatively slower at the lower concentration (Sander and Pignatello, 2005b). We have also shown that the normalized desorption rate increases in the presence of a competing co-solute, although it was not possible to apportion the rate increase between thermodynamic and kinetic effects (Zhao et al., 2002).

3.3 Strongly Resistant Desorption

Since the late 1980s there have been many published reports demonstrating biological effects that are apparently controlled by sorption-desorption kinetics (e.g., Pignatello and Xing, 1996; Alexander, 1995, 2000).

First, compounds that do not equilibrate rapidly with soil (e.g., polycyclic aromatic hydrocarbons) become less bioavailable to an organism (e.g., bacteria or earthworms) as the soil-chemical pre-contact time in the absence of the organism increases. This is due to continued diffusion of the contaminant to sites in the soil particles further and further removed from contact with the bulk fluid, where it becomes more and more resistant to desorption once the organism is introduced.

Second, contaminated soil or sediment collected from the environment often contains a fraction of the total extractable chemical present in a state that strongly resists desorption. In several cases a correlation exists between the desorption-resistant fraction and the fraction that is unavailable or much less available to a test organism.

Third, many studies examining sorption independently have shown that addition of a chemical to an initially clean soil often generates a minor fraction that strongly resists desorption. For example, Figure 7 shows the desorption profiles of phenanthrene from six different soils (Braida et al., 2002). Nine other soils showed similar behavior. The soils were pre-equilibrated with phenanthrene for 180 days under sterile conditions prior to initiating desorption. Uptake rate studies indicated apparent equilibrium (leveling off of the solution-phase concentration) occurred well within this timeframe. Desorption was carried out in the presence of Tenax polymer (beads) which rapidly and efficiently takes up desorbed molecules and, thus, serves as an infinite sink (Zhao and Pignatello, 2004). The Tenax was replaced at each desorption step, ensuring the steepest possible gradient in phenanthrene concentration across the soil-bulk water interface and, thus, the maximum rate of desorption. Except in the case of Mount Pleasant silt loam, a substantial fraction of phenanthrene resisted desorption after 500-600 days of desorption to infinite dilution. This fraction, which ranged from about 5 to 30% of the initial amount added, was recovered by hot-solvent extraction as unaltered phenanthrene. The desorption resistant fraction decreased with increasing applied phenanthrene concentration.

Plausible mechanisms for formation of strongly immobilized fractions in soils include: (i.) chemisorption, (ii.) entrapment during particle synthesis, (iii.) diffusion into remote sites, and (iv.) post-sorption rearrangement of the local matrix leading to sterically hindered desorption.

Chemisorption includes formation of covalent bonds with organic matter or coordination complexes with metal ions in the solid. Covalent linkage is possible if the sorbate undergoes chemical or biological transformation, and

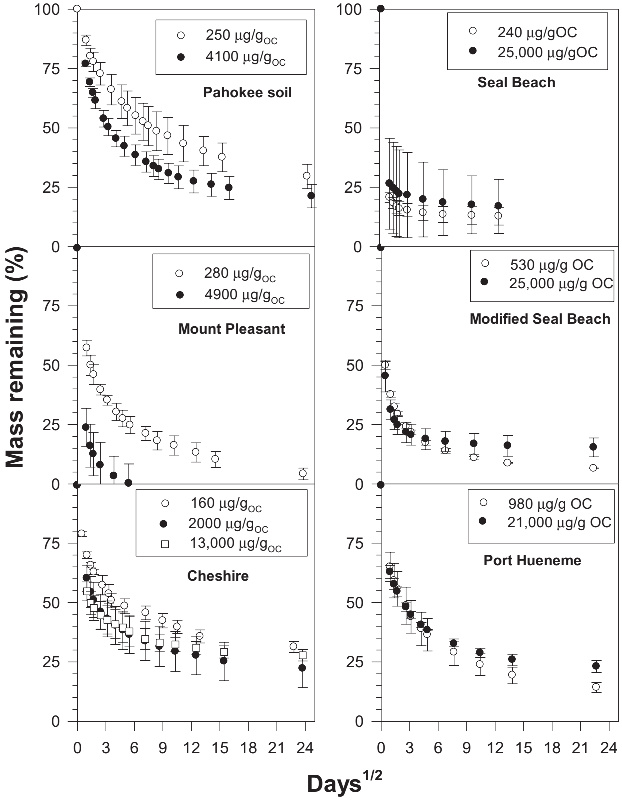


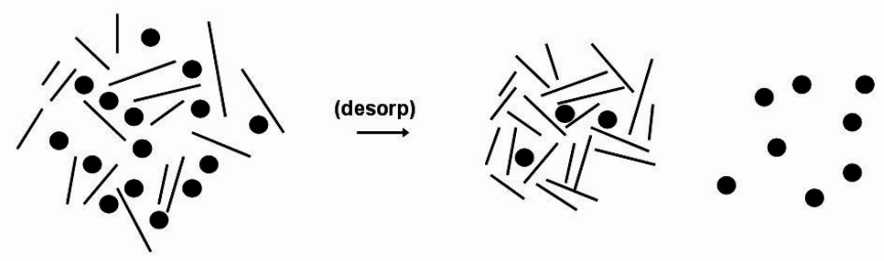
Figure 7. Desorption profiles of phenanthrene after 180 days pre-equilbration in soil-water suspension at indicated initial concentrations. Error bars represent cumulative uncertainty (95% level) of three to four replicates. Reprinted with some modification from Braida et al. (2002) with permission from the Society of Environmental Toxicology and Chemistry.

many examples of such reactions have been reported. In most cases, however, covalent bond formation leads to permanent loss of compound identity, even if any of the original atoms ultimately were to become bioavailable. Thus, covalent coupling cannot be said to lead to "desorption resistance." Coordination complexes require certain functional groups (e.g., a carboxyl or sulfhydryl) capable of forming inner-sphere bonds with the metal. Dissociation can be considerably slower than association. However, it should be realized that desorption resistance is broadly applicable, applying to compounds that do not contain any interacting functional groups whatsoever.

Entrapment during particle synthesis likely contributes to desorption resistance of PAHs in black carbon particles. Sediments rich in black carbon often contain a fraction of PAH compounds in a highly immobile state. Since PAHs are co-formed with soot during combustion of fuels, their apparent immobilization may result from entrapment in closed micropores during particle formation. Organic solvents are known to swell black carbon (Jonker and Koelmans, 2002; Braida et al., 2003), so such molecules can be counted as part of the total PAH because they would be liberated during analytical extraction.

Molecules may also be rendered desorption resistant if a fraction of them diffuse into sites at the ends of especially tortuous paths. In that case, transport to or from such sites would require much longer times than to or from more available sites. This is the classical explanation for desorption resistance. It is not hard to imagine the existence of such sites. Small bits of organic matter may be occluded within mineral aggregates (Kleineidam et al., 1999). Within the organic matrix there may be micro-domains that for steric reasons require longer times to access.

The last mechanism for sorbate immobilization (iv) requires a mechanism for matrix rearrangement. Random thermal motions of natural organic matter macromolecules do, of course, occur constantly, and some of these motions might lead to encagement of sorbed molecules that happen to be there. The same random motions could lead to their release, so one would expect to reach a steady state of a small fraction of temporarily-entrapped molecules. Kan et al. (1997) proposed that a fraction of sorbate molecules is transferred to "high-affinity" micro-environments "in equilibrium with the dissolved state" that are created by virtue of "conformational or physical rearrangement of a small fraction of the organic matter." However, this model was silent about the driving force for rearrangement and the underlying cause of enhanced affinity. Weber at al. (2002) observed strong hysteresis of the phenanthrene sorption isotherm in soil and shale in the presence of high concentrations of a co-solute. They postulated that the co-solute caused swelling of the solid; when desorption was initiated by dilution of the liquid phase, the abrupt change in sorbed concentration of the co-solute caused the matrix to collapse and stiffen around phenanthrene molecules still remaining in the solid (anti-plasticization effect). However, other explanations are possible, such as reduced competition for adsorption sites by the now-diluted co-solute, a purely artificial effect (Sander and Pignatello, submitted for publication). A similar matrix collapse mechanism was invoked by Braida et al. (2003) to explain pronounced sorption hysteresis of benzene in a wood charcoal (Scheme 6). They showed conclusively that benzene caused swelling of the char particles, but they gave no direct evidence for matrix entrapment.



(Scheme 5)

The 'matrix collapse' hypothesis has now been given some credence by recent studies of 1,4-dichlorobenzene (DCB) sorption in two soils (Sander and Pignatello, submitted for publication). Isotope exchange in both the forward and reverse directions was followed at the bulk sorption and bulk desorption points at two widely-spaced points on the isotherm. The mass of isotopically-labelled ^{14}C-DCB added or removed was miniscule relative to the concentration of existing bulk DCB. In all cases, the label re-equilibrated to exactly the same place (i.e., same distribution coefficient within experimental error) as the bulk after 35 days, remaining so over more than 100 days total. From this result it could be estimated that at most 3% of sorbed molecules could have been rendered out of free exchange with the solution state after either the bulk sorption or bulk desorption steps. Next, the samples were desorbed in three 21-day extractions in the presence of Tenax, renewed at each step. These exhaustive desorptions left significant amounts of ^{14}C-DCB in the solid ranging from 1.7 to 18.6% of initial, as given in Table 1. The data in Table 1 are corrected for a minor amount of chemically- or biologically-transformed DCB formed during the lengthy experiment. Most ^{14}C-DCB was removed in the first Tenax extraction, with declining yields in the second and especially third Tenax extraction. The fraction finally remaining was considerably greater at lower of the two concentrations. The fraction finally remaining at the low concentration was well outside the limits of uncertainty determined in the isotope exchange experiment. This means that it is possible ^{14}C-DCB became entrapped as a result of physical collapse of the matrix induced by abrupt Tenax extraction. However, as 418 days had passed in the interim between the initial spike of ^{14}C-DCB and initiation of Tenax extraction it is possible that during this lengthy time these fractions had penetrated remote sorption sites, even though penetration to these sites could not be detected in 100-day isotope exchange experiments. Further work is required to assess this important mechanism.

Table 1. Fraction of total initial UL-14C-1,4-dichlorobenzene remaining after desorption in each of three 21-day Tenax extraction steps.

Step	Pahokee peat		Beulah-Zap	
	initially 2.34 g/kg	initially 0.042 g/kg	initially 6.21 g/kg	initially 0.19 g/kg
1	0.059 ± 0.003[a]	0.186 ± 0.0066	0.0951 ± 0.007	0.101 ± 0.004
2	0.0268 ± 0.0006	0.143 ± 0.007	0.036 ± 0.003	0.065 ± 0.004
3	0.017 ± 0.001	0.120 ± 0.007	0.0230 ± 0.0008	0.051 ± 0.003

[a] Mean and standard deviation from triplicate experiments. The mean values are corrected for a small amount of label not identified as ^{14}C-DCB after hot-solvent extraction of the solid and comparison of ^{14}C-DCB concentrations determined by gas chromatography and liquid scintillation counting.

4. FACILITATED BIOAVAILABILITY

Facilitated bioavailability refers to the ability of organisms to directly access sorbed molecules or to promote desorption by altering the properties of the soil particles or the surrounding liquid.

Many ways can be envisioned by which organisms could alter the chemistry of the fluid or even the properties of the solid. Particles entering the digestive or respiratory tracts of organisms may be subjected to desorption-inducing conditions such as, a) higher temperatures than the surroundings, b) aqueous fluids that may include dissolved biosurfactants (e.g., bile acids, Voparil et al., 2003) or competitive adsorbates, or c) abrasion that breaks up particles—for example, in the gizzards of birds. Microorganisms may exude biosurfactants that can increase the apparent dissolved concentration. Plant roots can affect soil solution chemistry. Addition of chelating agents like citrate, oxalate or pyrophosphate was found to enhance desorption of hydrophobic compounds such as PAHs from soil organic matter by solubilizing metal ions that are essential for cross-linking humic substances or bridging humic substances to mineral surfaces (Yang et al., 2001; Subramaniam et al., 2004). Solubilization of humic substances accompanied mobilization of PAHs. Some evidence suggests that exudation of chelating agents by plant roots may facilitate uptake of chlorinated hydrocarbon insecticides by this mechanism (White et al., 2003), although other explanations are possible. Natural organic acids exuded by plants can also plausibly increase bioavailability by competitively displacing contaminants from sorption sites (Xing and Pignatello, 1998).

Single-cell organisms may lie in close contact with soil surfaces and the question has arisen whether or not they may have "direct access" to molecules in the sorbed state. While some studies suggest they do not, others indicate that some microorganisms may have facilitated access to sorbed molecules (Guerin and Boyd, 1992; Crocker et al., 1995; Dean et al., 2001; Li et al., 2005). The mechanism of facilitated access is unclear at this time. Bacterial cells are on the order of 10^{-6} m in diameter; therefore, they cannot penetrate mesopores (2-50 nm) or micropores (< 2 nm), which contribute nearly all the surface area of soil particles. Nor can they penetrate the internal matrix of natural organic matter, where sorbed molecules of nonionic organic compounds are primarily located. While it is true that the concentration of a contaminant on the particle surface on an absolute scale may be orders of magnitude higher than it is in solution, the chemical potential is identical if local equilibrium is assumed (Pignatello, 2000). Thus, at a given solution concentration, the equilibrium uptake by cell membrane is independent of whether soil is present or not.

We have attempted to address the question of facilitated access of desorption-resistant residues by bacteria. Braida et al. (2004) spiked phenanthrene to 15 different sterilized soils and allowed them to equilibrate for 180 days. Then, in separate experiments each lasting 30 days, replicate flasks were amended with either–Tenax to initiate desorption—still under sterile conditions—or with an inoculum of a bacterial culture grown on phenanthrene as the sole C and energy source. Braida et al. (2004; their Figure 4) showed the relationship between the biotransformation-resistant fraction and the Tenax desorption-resistant fraction. Most of the points lie near the 1:1 line. Two or three soils show a significantly smaller desorption-resistant fraction than biodegradation-resistant fraction, indicating that biology, not desorption was rate-limiting. Only one soil shows the opposite result consistent with facilitated desorption. In another study of phenanthrene in two soils using two bacterial cultures, White et al. (1999) showed that the normalized desorption and biodegradation rates decreased with a similar slope with pre-test sterile aging period, as did total amount desorbed and total amount biodegraded within a specified period. The results of these studies (White et al., 1999; Braida et al., 2004) underscore the link between physical availability and biological availability and point away from the facilitated bioavailability hypothesis.

A different result was obtained in another study, however. That study examined biodegradation by native microorganisms of a set of 15 PAH compounds in coal-tar residues in the soil surrounding a former manufactured gas plant (MGP) in Connecticut, U.S.A. (Li et al., 2005). Samples were suspended in deionized water and incubated under non-sterile conditions allowing the native organisms to degrade the PAHs. Desorption was monitored in separate sterile flasks containing Tenax and including 0.01 M citrate in solution, which was added to maximize the rate of desorption (Yang et al., 2001; Subramaniam et al., 2004). After about 100 days in each case, the remaining PAH was quantified. The results (Figure 8) show scatter about the 1:1 line defining the relationship between biodegradation resistance and desorption resistance consistent with no facilitated bioavailability, and concordant with the studies of Braida et al. (2004) and White et al. (1999), among others. In some experiments, a suite of macro- (NH_4^+, P, K) and micro- (Mg, Ca, Fe, Co, Zn, Cu, Ni, Mo, Se, W) nutrients was included in the live flasks. Nutrient amendment was found to greatly stimulate biodegradation of PAHs in this coal tar soil. When nutrients were included, the biodegradation-resistant fraction was found to be generally smaller than the desorption-resistant fraction (Figure 9). This result suggests that microorganisms can, indeed, facilitate desorption.

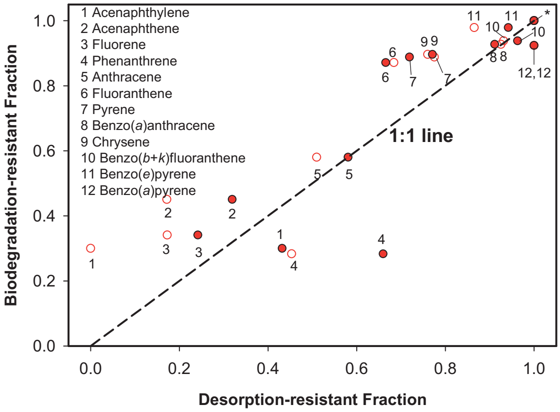


Figure 8. Comparison of biodegradation-resistant fraction (99 days) and desorption-resistant fraction (after 106 days) of PAHs in a coal tar contaminated soil. Biodegradation was carried out in soil suspension in deionized water and no supplemental culture of microorganisms. Desorption was carried out in 0.01 M citrate (open symbols) or 0.01 M pyrophosphate (filled symbols) solution and in the presence of Tenax beads to extract desorbed PAHs. Data from Li et al., 2005.

It appears that the persistence of PAHs for many decades at this MGP site is due, in part, to nutrient limitations of the native microorganisms. Exactly which nutrients are limiting is unknown. Had we not included nutrients in the experiment we would have falsely concluded no facilitated bioavailability.

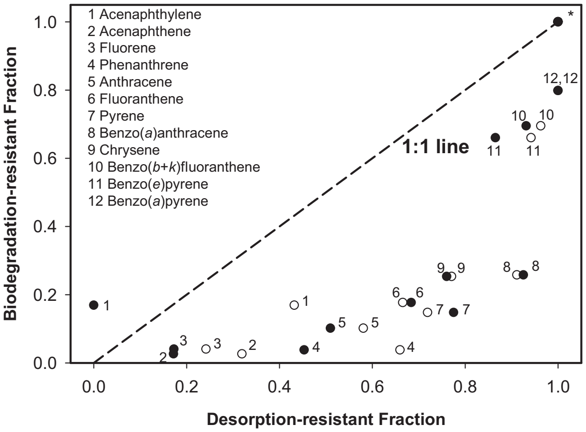


Figure 9. Same as Figure 8 except live samples contained a mixture of inorganic nutrients. Li et al., 2005; reprinted with permission from the Society of Environmental Toxicology and Chemistry.

The condition that distinguishes the coal tar case (Li et al., 2005) from the phenanthrene cases (Braida et al., 2004; White et al., 1999) is that the former followed substrate degradation by existing native organisms, while the latter two used enrichment culture amendments. (All experiments included nutrient amendment.) Thus, it is possible that native phenotypes possess unique adaptations that allow them to access pollutant mass that is unavailable to other organisms. It is also possible that the colonies are essentially different in the two experimental systems: the degraders in the coal tar soil contained established biofilms while the degrader inoculum in the phenanthrene-spiked soils was initially present as free-living cells (the degree of cell attachment was undetermined). It is possible that established biofilms extend deeper into pore structures, thus steepening the concentration gradient at the interface between the external surfaces of the particle and the bulk water. Further work is necessary to clarify the mechanism of facilitated bioavailability for microorganisms.

5. SUMMARY

Sorption to particles in soil or sediment determines the immediate biological availability of chemicals to potentially affected organisms, the dispersion of contaminants within an ecosystem, and the transport of contaminants to other ecosystems. Thus, sorption plays a role in the assessment of present and future risks to humans and ecosystems, and often enters into strategic decisions about remediating a contaminated site. The focus of this paper was on the role of sorption in regulating bioavailability.

The influence of sorption thermodynamics has been evaluated by considering equilibrium partitioning between soil, water and biomembrane phases. The Biomembrane Soil Organic Carbon Concentration Factor ($BS_{OC}CF$) may be defined as the ratio of biomembrane-water to soil OC-water distribution coefficients. Based on abundant sorption data and limited data on partitioning of chemicals in liposomes as model membranes one can make predictions about equilibrium bioavailability. $BS_{OC}CF$ is predicted to increase with contaminant concentration or the concentration of a competing co-solute due to the typically nonlinear nature of sorption to soil OC. Bioavailability enhancement by a competing, non-metabolized co-solute is validated experimentally for microorganisms. However, at constant concentration, the $BS_{OC}CF$ is predicted to be only weakly dependent on solute polarity or hydrophobicity indices.

Sorption exhibits “irreversible” phenomena, including hysteresis and conditioning effects caused by irreversible pore expansion of the glassy natural organic matter solid phases by the penetrating chemical. Irreversible behaviors are expected to result in a decrease in $BS_{OC}CF$. Because irreversible sorption always results in enhanced sorption affinity in a subsequent experiment, it is predicted to result in a decrease in equilibrium bioavailability.

The influence of sorption kinetics is discussed for circumstances in which desorption from particles is rate limiting. The characteristic sorption/desorption rate parameter (or normalized rate of uptake or release) decreases with solute molecular size, increases with solute concentration, and increases with concentration of a competing co-solute. Collected field samples often contain a strongly desorption-resistant fraction. Also, such a fraction may be generated when a contaminant is added to a clean sample. Mechanisms for sequestration include entrapment by co-condensation during particle synthesis (i.e., PAHs in soot particles), diffusion to ‘remote’ sites, and matrix collapse following sorption. The matrix collapse mechanism is a possible outcome of the irreversible pore deformation mechanism of sorption irreversibility and is a result of swelling-shrinking processes caused by the contaminant itself.

Some studies support the hypothesis of facilitated bioavailability in which cells either have access to sorbed molecules or are able to promote desorption by altering the chemical or physical properties of the soil particle or surrounding liquid. In studies of phenanthrene degradation with added cultures we have found little support for facilitated bioavailability. However, in a study of PAH degradation by native organisms in a coal tar contaminated soil we observed a markedly smaller biodegradation-resistant fraction than the corresponding desorption-resistant fraction when biodegradation was stimulated with inorganic nutrients. This suggests that facilitated bioavailability is possible for adapted native populations when nutrient limitations are absent.

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THE ROLE OF HUMIC SUBSTANCES IN THE FATE OF ANTHROPOGENIC ORGANIC POLLUTANTS IN SOIL WITH EMPHASIS ON ENDOCRINE DISRUPTOR COMPOUNDS

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Abstract: In the first part of this paper an overview is provided of the various phenomena that anthropogenic organic pollutants (AOPs) of various nature and origin are subjected in soil and of the specific role exerted by soil humic substances (HS) in these processes. The paper then focuses on adsorption/desorption of an important class of organic pollutants, i.e., endocrine disruptor compounds (EDCs), onto soil humic acids (HAs) that are the most abundant and chemically and biochemically active fractions of HS and soil organic matter. EDCs are hormone-like substances that include natural and synthetic estrogens and several pesticides, industrial chemicals, and plastic and pharmaceutical products and byproducts which are able to alter, i.e., disrupt, the normal endocrine functions in animals and humans. These compounds may enter the soil through current agricultural practices and /or disposal of urban and industrial effluents, sludges and wastes. The estrogenic risk of EDCs is generally related to their distribution and speciation in the various soil phases, in which adsorption/desorption processes play a very important role. Adsorption kinetics and adsorption/desorption isotherms of the EDCs bisphenol A (BPA), octylphenol (OP), 17-alpha-ethynyl estradiol (EED) and 17-beta-estradiol (17ED) onto HA samples isolated from the surface (0-30 cm) and deep (30-90 cm) horizons of two sandy soils were determined using a batch equilibrium method and the HPLC technique. Adsorption of EDCs onto HAs occurs in two phases, a rapid one in the first few hours of contact, which corresponds to more than 90% of total adsorption, and a slow one that needs generally less than 24 h to be completed. Experimental adsorption data were best fitted in a Langmuir isotherm for BPA and 17ED, in a linear isotherm for OP and in a nonlinear, L-shaped Freundlich isotherm for EED. Thus, no limiting adsorption is observed for EED and OP

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onto HAs examined over the concentration range tested, whereas a maximum adsorption, i.e., saturation, is reached only by BPA and 17ED. In general, the adsorption capacity for EDCs of HAs from surface horizon soils is much larger than that of HAs from deep horizon soils. The extent of adsorption of BPA is generally smaller than that of the other three EDCs that are adsorbed at almost the same extent. Adsorption of EED and, especially, BPA onto HAs is generally reversible, and desorption occurs quickly and almost completely after few desorption steps. On the contrary, adsorption of OP is mostly irreversible, a partial desorption occurs slowly, and high amounts of OP are retained by most HAs at the end of the experiment. In conclusion, the soil HAs examined in this work are able to adsorb variable amounts of EDCs that tend to accumulate in the soil surface horizon. EDCs quickly and completely desorbed are expected to move easily down the soil profile, and possibly contaminate groundwater, whereas EDCs slowly and only partially desorbed are expected to remain mostly HA-adsorbed, especially on the surface soil layer, with corresponding top soil contamination.

Key words: Anthropogenic organic pollutants; soil; humic substances; endocrine disruptor compounds; adsorptio/desorption isotherms

1. INTRODUCTION

An “anthropogenic organic pollutant” (AOP) may be defined as “any organic chemical that is foreign to the natural ecosystem and may adversely affect, either directly or indirectly, the natural physical, chemical and biological equilibria and processes in the global environment or a portion of it”, e.g., the soil.

The purpose of this papers is to firstly provide an introductory overview of the various phenomena that AOPs of various nature and origin are subjected in soils and on the specific role exerted by soil humic substances (HS) on these processes. Thus, the general and succinct concepts and information contained in sections 2 to 4 are meant to be addressed to those readers who are not familiar with AOP behaviour and fate in soil and related processes involving soil HS. For this reason, and due to the limited space available for this part of the text, detailed references are not included but only some fundamental and comprehensive reviews and books are cited to which the interested reader may refer for further details. The paper will then focus on adsorption/desorption of an important class of organic pollutants, i.e., endocrine disruptor compounds (EDCs), onto soil humic acids (HAs) that are the most abundant and chemically and biochemically active fractions of HS and soil organic matter.

Adsorption/desorption of EDCs onto soil HAs is generally considered one of the most important processes that controls EDC behavior, performances, and fate in soil, including mobility, transport, accumulation, bioavailability and toxicity, and the assessment of their potential environmental hazard.

2. ANTHROPOGENIC ORGANIC POLLUTANTS AND THEIR FATE IN SOIL

Substantial amounts of AOPs can reach the soil either by purpose, e.g., pesticides, or incidentally, i.e., deposited from a variety of sources that include wet and dry atmospheric deposition of AOPs originated from waste chemicals and solid waste incineration, introduced into the soil by discharge of municipal and industrial wastes, refuses, sludges and effluents, also used as soil amendement.

Potential AOPs that can reach the soil include chemicals of widely differing classes and properties. The most important and abundant of these are: (a) various types of pesticides, e.g., herbicides, insecticides, fungicides, etc., currently used in large amounts in common agricultural practices and agricultural product conservation, which belong to various classes of organic compounds of extremely variable physical and chemical properties; (b) polynuclear aromatic hydrocarbons (PAH) present in waste streams originated from various industrial processes such as the combustion of fossil fuels, chemical manufacturing, petroleum refining, metallurgical processes, and some coal, oilshale and tar sand conversion systems; (c) phtalic acid diesters (PAE) used mainly as plasticizers, but also as pesticide carriers and insect repellents, in dyes, cosmetics and lubrificants; (d) endocrine disruptor compounds (EDCs) (see below); and (e) several alkanes, organic solvents, detergents, and surfactants (Senesi, 1993a).

Once on soil surface, the parent AOPs and their degradation products may be subjected to various fates (Figure 1). They can be transported by soil runoff and/or erosion to surface waters, or undergo volatilization and/or photodecomposition. They can enter the soil and be leached, eventually reaching the groundwater table, or be subjected to various phenomena including partial or total chemical decomposition and/or biological degradation, uptake by plant roots, and various physical and chemical interactions with soil inorganic and organic solid and colloidal components (Senesi, 1993a; Senesi and Miano, 1995).

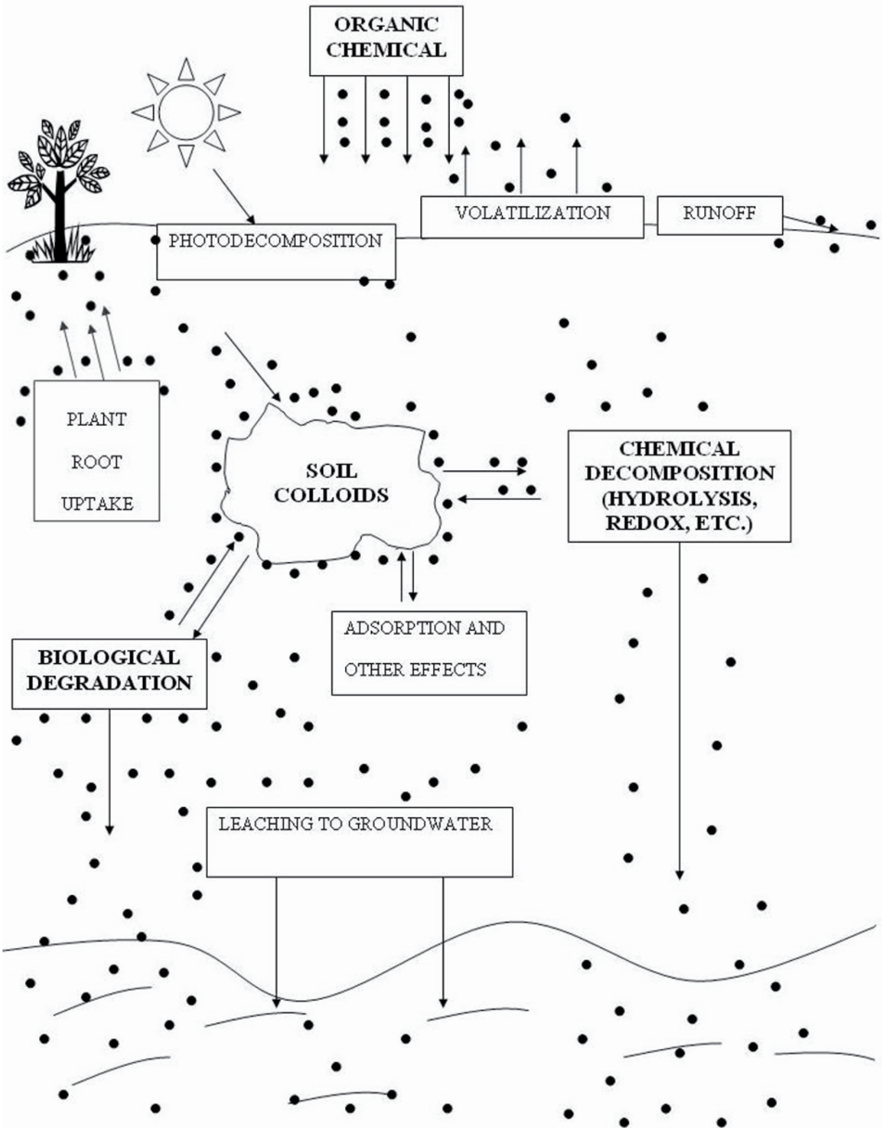

Figure 1. Fate of organic pollutants in soil.

The behaviour and performance of AOPs in soil is controlled by several factors including the physical and chemical properties of the AOP, the thickness, hydrological status and nature of soil, and the type and extent of interactions of the AOP with the various inorganic, organic and biological soil components. Most studies have suggested that AOPs show affinity for soil organic colloids greater than that for mineral colloids. Thus, the content and nature of soil organic matter, and especially its HS fractions, play a major role in determining the fate of AOPs in soil (e.g., Stevenson, 1994; Senesi and Miano, 1995).

3. SOIL HUMIC SUBSTANCES

Soil HS consist of a physically and chemically heterogeneous mixture of relatively high-molecular-mass, yellow-to-black colored organic compounds of mixed aliphatic and aromatic nature, formed by secondary synthesis reactions, i.e., humification, of products of microbial and chemical decay and transformation and recalcitrant residues of biomolecules originated from organisms during life and after death (e.g., Stevenson, 1994). These materials are exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass.

Approximately 60-70% of the total soil organic carbon occurs in HS. The estimated level of soil organic carbon on the earth surface occurring as HS is 30 x 10^{14} Kg (Stevenson, 1994). As such, HS are the most ubiquitous and widespread natural non-living organic materials in soils, as well as in all terrestrial and aquatic environments.

On the basis of their solubility in water solutions at various pH, HS are divided into two main fractions, that are: (a) humic acids (HAs), the portion that is soluble in dilute alkaline solution and is precipitated upon acidification to pH 2; and (b) fulvic acids (FAs), the portion that is soluble at any pH values, even below 2 (e.g., Stevenson, 1994). HAs and FAs cannot be regarded as single chemical individuals described by unique, chemically defined molecular formulas. Rather, they can be operationally described by model structures constructed on the basis of available compositional, structural, functional and behavioral data and containing the same basic structural units and the same type of reactive functional groups that are common to all the single, indefinitely variable and unknown molecules (e.g., Stevenson, 1994).

Figure 2 illustrates one example of a “typical” model macromolecule of soil HA which basically consists of aromatic, phenolic, quinonic and heterocyclic “building blocks” that are randomly condensed or linked by aliphatic, oxygen, nitrogen, or sulphur bridges. The macromolecule bears aliphatic, glucidic, aminoacidic and lipidic surface chains as well as chemically reactive functional groups of various nature (mainly carboxylic and phenolic but also alcoholic hydroxyls, carbonyls, etc.) which render the HA polymer acidic. The structure and composition of FAs are less complex than those of HAs. FAs generally feature a lower molecular mass and aromaticity and higher solubility, aliphatic character and content of O-containing functional groups.

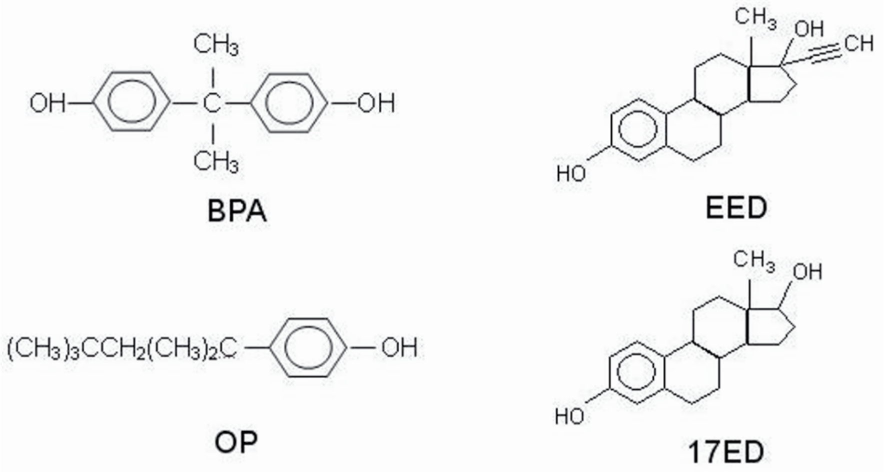

Figure 2. Molecular formulas of endocrine disruptor compounds examined: bisphenol A (BPA), Octylphenol (OP), Ethynilestradiol (EED), and 17 B estradiol (17ED).

HS are rich in hydrophilic and hydrophobic sites, exhibit a polydispersed and polyelectrolitic character, possess surface activivity, and present a relatively open, flexible, sponge-like structure rich of holes (Schnitzer, 1978; Hayes and Swift, 1978). An additional typical feature of HS is the presence in their structure of highly reactive organic free radical moieties of prevalent semiquinonic nature (Senesi, 1990).

All the above described properties qualify HS, and especially HAs, as privileged natural organic compounds in the interaction with AOPs.

4. INTERACTIONS BETWEEN HUMIC SUBSTANCES AND ORGANIC POLLUTANTS

HS may interact with AOPs in several ways and thus influence their behavior, performance and fate in soil with important implications in their persistence, imobilization and accumulation, mobility and transport, biovailability and biotoxicity, degradability, volatilization and leaching, and AOP residue monitoring in soil. In particular, HS have been shown to be able to modify water solubility of AOPs, exert catalytic activity on some AOP transformations, act as photosensitizers promoting AOP photo-degradation, and, especially, adsorb and partition AOPs (e.g., reviews by

Senesi and Chen, 1989; Senesi, 1992, 1993 a, 1993b; Senesi and Miano, 1995).

In the presence of HS, relatively water-insoluble, nonionic AOPs, e.g., PAHs, PAEs, PCBs, DDT, n-alkanes, are mostly easily solubilized, possibly as a result of AOP partitioning into HS or adsorption onto HS, or of an overall increase in solvency. HAs have been shown to enhance water solubility of DDT and PCBs more effectively than FAs, possibly due to their larger molecular mass and nonpolar volume. The magnitude of AOP solubility enhancement was also shown to be dependent on the concentration of HS, pH and temperature. For example, water solubility of DDT and some PCBs increased at low HS concentrations and by a decrease of temperature, whereas it decreased by a raise in pH.

HS, especially in the dissolved phase, were shown to exert a positive or negative catalytic effect in the abiotic hydrolysis of some pesticides. For example, the acid hydrolysis rate of the chloro-s-triazines simazine, atrazine and propazine was enhanced in the presence of FAs or HAs. This effect was possibly attributed to the specific H-bonding interaction occurring between surface carboxylic groups of the HS molecule and the side-chain N atoms of the triazine, which would promote replacement of the Cl atom by water. On the contrary, the alkaline hydrolysis rate of n-alkyl esters of 2,4-D (2,4-DOE) was lowered in the presence of FAs or HAs. Inhibition by HAs of hydrolytic enzymes in soil was indicated as an additional possible mechanism that may operate in certain reactions.

HS are able to adsorb sunlight, and thus behave as initiators of photoreactions, e.g., as photosensitizers, of some AOPs in the top layer of soil by accelerating, increasing or even determining light-induced transformation of AOPs. HS principally act as precursors for the production of greatly reactive, short-lived photoreactants such as the solvated electron, e^{-}_{aq}, singlet oxygen, $^{1}O_2$, superoxide anion, O_2^{-}, peroxy radicals, $RO_2^{\cdot}$, hydrogen peroxide, H_2O_2, and redox-active species including photoexcited parent HS molecules and HS organic radicals. However, HS can also act as scavengers of other phototransients such as the hydroxyl radical, $OH^{\cdot}$. Photodegradation may modify the physical and chemical properties of AOPs, and significantly affect their fate and migration into the bulk soil. For example, photolysis of atrazine was shown to be more extensive in the presence of FA.

Adsorption represents probably the most important mode of interaction of AOPs with soil components, and especially with HS. AOPs can be adsorbed to various extent by HS through specific physical and chemical binding mechanisms and forces with varying degrees and strenghts. These include ionic, hydrogen and covalent bonding, charge-transfer or electron donor-acceptor mechanisms, dipole-dipole and Van der Waals forces, ligand exchange, and cation and water bridging. However, adsorption of nonpolar (hydrophobic) AOPs can be better described in terms of non-specific, hydrophobic or partitioning processes between soil water and the HS organic phase.

The mechanism and extent of adsorption will depend on the amount and the physical and chemical nature and properties of both the AOP and the HS. These include: the molecular structure, the number and type of functional groups, the size, shape and configuration, the polarity, polarizability and charge distribution, and solubility of both HS and AOP, and the acidic or basic or neutral, ionic or nonionic, polar or nonpolar nature of AOP. The conditions of the medium, such as pH, ionic strength, redox potential, amount of water, will also greatly influence adsorption of AOPs onto soil HS.

The formation of ionic bonds is generally achieved following proton transfer from acidic carboxylic and phenolic OH groups (proton donors) of HS macromolecules and appropriate proton acceptor basic groups of the AOPs such as the pesticides diquat, paraquat, chlordimeform, phosphon, phenacridane chloride, s-triazines and amitrole. Several O- and N-containing sites available on HS macromolecules can be used to form hydrogen bonds with several AOPs containing suitable complementary groups, such as acidic pesticides, including chlorophenoxyalkanoic acids and esters, asulam and dicamba, and several nonionic polar pesticides, including substituted ureas, phenylcarbamates, alachlor, metolachlor, cycloate, malathion and glyphosate. HS macromolecules contain both electron-deficient structural moieties, such as quinones, and electron-rich moieties, such as diphenols, which result in the formation of electron donor-acceptor, or charge-transfer, bonding with AOPs possessing the complementary feature, i.e., electron donor groups, such as s-triazines, substituted ureas, amitrole, or electron acceptor groups, such as paraquat, diquat, chlorodimeform, chloranyl, DDT, PCBs, dioxins. The formation of stable covalent bonds, often mediated by chemical, photochemical and enzymatic catalysts, has been shown to occur between reactive functional groups of HS and suitable functional groups of AOPs such as chlorophenoxyalkanoic acids and esters, and their degradation products such as anilines, catechols and phenols. HS have been shown to be able to adsorb s-triazines and anionic pesticides, such as pichloram, through ligand exchange mechanisms occurring through the displacement of hydration water or other weak ligands partially holding a polyvalent metal ion associated to HS by a suitable functional group ligand of the AOP molecule. Dipole-dipole and Van der Waals forces are considered also to occur in AOP adsorption onto HS, either in addition to stronger binding forces or, more important, in the adsorption of nonionic and nonpolar AOPs. Finally, hydrophobic adsorption, or partitioning, on HS hydrophobic active sites, such as aliphatic side chains and lipidic and aromatic structural moieties, has been shown to be an important nonspecific mechanism acting for nonionic, nonpolar AOPs that interact weakly with water, such as DDT and other organochlorine insecticides, PAEs, PAHs, PCBs, etc.

For any given AOP, several possible adsorption mechanisms may operate simultaneously and/or in sequence. Initially, the AOP molecule may be adsorbed by HS sites providing the strongest binding, followed by progressively weaker sites as the stronger sites become filled. Once adsorbed, the AOP may be subject to other processes, e.g., may further react becoming either covalently and irreversibly bound or only physically trapped into the HS matrix. Adsorption processes may thus vary from complete reversibility to total irreversibility, i.e., the adsorbed AOP may be easily desorbed, desorbed with various degrees of difficulty, or not at all. Further, the effect of adsorption on AOP migration in soil depends on whether the adsorption occurs on insoluble, immobile HS such HAs, or on dissolved or suspended, mobile fractions such as FAs. Thus, HS can either "attenuate" or "facilitate" AOP movement and transport in soil.

A comprehensive study of adsorption/desorption processes of pesticides onto HS should include the evaluation of quantitative aspects, through the measurement of adsorption/desorption isotherms and coefficients (see below for EDCs), and qualitative and molecular aspects through the determination of adsorption/desorption mechanisms. More detailed information and discussion on the above topics can be found in several recent reviews (e.g., Senesi and Chen, 1989; Senesi, 1992, 1993 a, 1993b; Senesi and Miano, 1995).

5. ENDOCRINE DISRUPTOR COMPOUNDS

Several organic compounds of natural and anthropogenic origin are suspected or known to interfere with the endocrine system of animals and humans by acting as hormone-like substances in the organism (Lintelmann et al., 2003). These compounds are known as "endocrine disruptor compounds" (EDCs), and possess the property to alter or disrupt the normal functioning of the endocrine system either directly, by blocking or imitating natural hormones, or indirectly, by interfering with the synthesis, storage, secretion, transport, catabolism and activity of various natural hormones (Kavlock et al., 1996). Although the consequences of such interference on animal and human health are not yet completely understood, it is known that small disturbances in endocrine functions, especially during certain stages of life, such as development, pregnancy, and lactation, can lead to serious and long-lasting effects (Colborn and Clement, 1992). Recent progress in both epidemiological and toxicological research indicates that exposure to potentially endocrine disrupting agents may interfere with human reproductive function and success, and that these agents may have a particularly important role in the aetiology of a variety of endocrine mediated disorders (Nolan, 1998).

Compounds proven or suspected to act as EDCs may be often introduced into the environment through common agricultural practices and by application, discharge and/or disposal of sewage and industrial effluents and sludges, and disposal of plastic and pharmaceutical residues. Different classes of herbicides, fungicides, insecticides and nematicides, several industrial chemicals, including PCBs, PAEs, dioxins, and some pharmaceutical products, like estrogenic compounds, have been identified as potential environmental EDCs (Colborn et al., 1993). Despite the high inputs and potential toxicity of EDCs, and the increasing social concern for a reliable risk assessment of the actual exposure of animals and humans to EDCs, relatively few data are available on the types and amounts of EDCs introduced into soil and aquatic systems. Until now, the attention on this issue has mainly focused on water contamination, and very few information is available on soil contamination by EDCs.

The risk assessment of potential environmental hazards associated with the presence of EDCs in soil, as well as the definition of possible remediation measures, require an accurate evaluation and quantification of the soil response to these compounds. In particular, the study of adsorption/desorption processes of EDCs onto soil HAs represent a key factor for the understanding of the fate of EDCs in soil. The objective of this work was thus to determine adsorption kinetics and adsorption and desorption isotherms of some representative EDCs onto some soil HAs.

6. MATERIALS

The EDCs used in this study were the following: bisphenol A (BPA) [2,2-(4,4-dihydroxydiphenyl)propane], octylphenol (OP) [4-(1,1,3,3-tetramethylbutyl)phenol], ethynyl estradiol (EED) [17alpha-ethynil-1,3,5(10)-estratriene-3,17beta-diol], and 17-beta-estradiol (17ED) [1,3,5(10)-estratriene-3,17beta-diol]. The molecular formulae of these EDCs are shown in Figure 3.

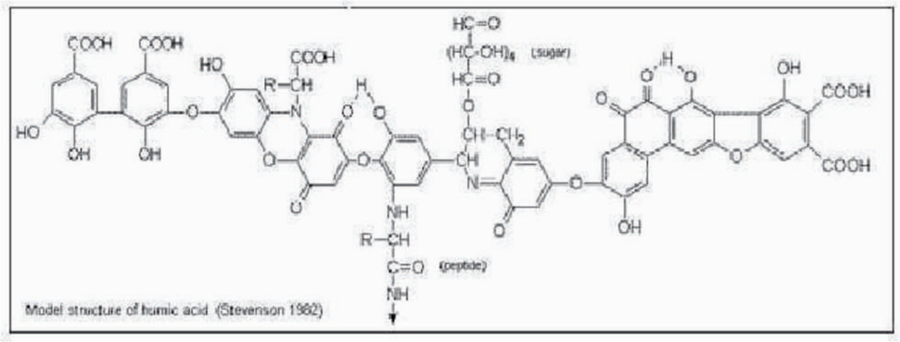

Figure 3. Model structure of humic acid.

The HA fractions of soil organic matter used in this study were isolated by a conventional procedure (Schnitzer, 1982) from two horizons (0-30 cm and 30-90 cm deep) of two different sandy soils sampled near Lisbon (Portugal) and Dresden (Germany), which are abbreviated, respectively, P30 and P90, and G30 and G90. Briefly, each soil sample was extracted three successive times by a 0.5 M NaOH + $Na_4P_2O_7$ solution. After centrifugation, the supernatants were unified and acidified with HCl to pH 1-2. The precipitated HA was separated by centrifugation, then purified by two alternate alkaline dissolution and acid precipitation treatments, and finally freeze dried to obtain a fine-powdered HA sample.

The four HA samples were characterized chemically and physico-chemically by current analytical methods and techniques (Senesi and Loffredo, 1999) for moisture and ash contents, yield, elemental composition, total acidity, carboxyl and phenolic hydroxyl group contents, the ratio of absorbances at 460 and 660 nm (E_4/E_6 ratio), organic free radical (OFR) content, and by various spectroscopic techniques.

7. METHODS

7.1 Adsorption Kinetics

Aliquots of 10 mg of each HA were suspended either in 5 mL of a 10 mg/L aqueous solution of BPA, or 25 mL of a 1 mg/L 6% (v/v) ethanol/water solution of OP, or 15 mL of a 10 mg/L 5% (v/v) ethanol/water solution of EED or 17ED. The pH of HA suspensions so obtained ranged from 4.4. to 4.9. The mixtures were mechanicaly shaken for 0.25, 0.5, 1, 2, 4, 8, 16, 24, 48, and 72 h, and centrifuged. The supernatant solutions were then analyzed by high performance liquid chromatography (HPLC) to determine the concentrations of each EDC using the same procedure as for determining adsorption isotherms as described below. All experiments were conducted in triplicate at a temperature of 20+/-2°C.

7.2 Adsorption Isotherms

Adsorption isotherms of each EDC onto each HA were obtained using a batch equilibrium method. Aliquots of 10 mg HA were added in glass flasks to 5 mL of aqueous solutions of BPA at concentrations of 1, 2, 4, 8, 12, 20 and 40 mg/L, and to 15 mL of 10 % (v/v) ethanol/water solution of OP or 5% (v/v) ethanol/water solution of EED or 17ED at concentrations of 0.1, 0.2, 0.5, 1, 2 and 5 mg/L. All experiments were conducted in triplicate. Equilibration was achieved by mechanical shaking of mixtures for 24 h at 20+/-2°C in the dark. Suspensions were centrifuged at 17,400 g for 15 min, and the supernatant solutions were then analyzed for the equibrium concentrations, C_e, of each free EDC by HPLC. A Thermo Separation Products Liquid Chromatograph was used equipped with a Merk LiChrospher® 60 RP-Select B column for BPA, EED and 17ED, and a SUPELCOSIL™ LC-18 column for OP. In all cases, the mobile phase used was a solution of acetonitrile/water at a ratio of 40/60 (v/v) for BPA, 50/50 (v/v) for EED and 17ED, and 75/25 (v/v) for OP. Ultraviolet (UV) detection at 280 nm was used for BPA, and fluorescence detection at 230 nm excitation and 310 nm emission for OP.

In the case of EED and 17ED, 2-mL aliquots of the supernatant solutions were subjected to a solid phase extraction (SPE) procedure using a Merk Lichrolut Extraction Unit and Merk Lichrolut EN cartridges (200 mg). The cartridges were previously conditioned with 2 mL of methanol and then with 2 mL of 5% (v/v) methanol in water. After the addition of sample solution, the cartridge was dried under vacuum, and the residue eluted twice 1 mL with acetone. Eluates were then analyzed by HPLC using a fluorescence detector operating at excitation and emission wavelengths of 280 and 306 nm, respectively.

The amount of each EDC adsorbed was calculated as the difference between the initial and the equilibrium amounts of EDC in solution. To construct adsorption isotherms, experimental adsorption data of each EDC onto each HA were fitted tentatively to both a linear model and a nonlinear Freundlich equation

$$x/m = K_f\, C_e^{1/n} \tag{1}$$

and a Langmuir equation

$$x/m = (KbC_e)/(1 + KC_e) \quad (2)$$

where x/m is the amount of EDC adsorbed in µg/g, and C_e is the equilibrium concentration of EDC in solution in µg/mL. The magnitude of adsorption, i.e., the adsorption capacity of the HA substrate, was estimated by the values of the Freundlich constant, K_f, and the distribution coefficient, K_d, which was calculated at each equilibrium concentration as the mean value of the ratios of sorbed concentration to the solution-phase concentration at equilibrium.

7.3 Desorption Isotherms

Desorption isotherms of BPA, OP and EED from each HA were obtained by measuring the sequential release of each compound immediately after its adsorption onto 10 mg of HA. This was achieved using either 5 mL of a 20-mg/L aqueous solution of BPA, or 15 mL of a 5 - mg/L of 10 % (v/v) ethanol/water solution of OP, or 15 mL of a 2 - mg/L 5% (v/v) ethanol/water solution of EED. Attempts made to measure desorption of 17ED from substrates examined were unsuccessful, because 17ED apparently degraded during the experiments, as shown by the appearance of not quantifiable degradation products in the HPLC analysis data.

After 24-h equilibration, the mixtures were centrifuged, and the equilibrium solution was carefully removed and replaced with the same volume (4 mL for BPA and 14 mL for OP and EED) of doubly distilled water, in the case of BPA, or 10% (v/v) of ethanol/water solution in the case of OP, or 5% (v/v) ethanol/water solution in the case of EED. The amount of dissolved EDC present in the equilibrium solution that remain entrapped in the substrate was duly calculated and subtracted from the total amount of EDC measured in the supernatant solution after each desorption step. The suspensions were shaken mechanically for 24 h in order to obtain a new equilibrium condition, and then centrifuged. The desorption procedure for BPA was repeated five times or until its concentration in the supernatant solution reached the detection limit of 1 microg/mL, which corresponds to the lower limit of BPA amount detectable in the conditions used, five times for OP, and six times for EED. All experiments were conducted in triplicate.

After each desorption step, the concentration of EDC in the supernatant solutions was measured by HPLC as described above, and the amount of EDC that remained adsorbed was calculated by difference. For comparative purpose, desorption isotherm parameters, K_{fdes} and $1/n_{des}$, were calculated from the Freundlich equation (1) in all cases. The values of K_{fdes} provide an indication of the degree of irreversibility of the adsorption process

(McCall et al., 1981), whereas the values of $1/n_{des}$ account for nonlinearity of the desorption isotherm and is an index of the intensity of desorption (Pignatello and Huang, 1991.

8. RESULTS AND DISCUSSION

8.1 Humic Acids

The ash content, extraction yield, elemental and acidic functional group composition, E_4/E_6 ratio, and concentration of organic free radicals (OFR) of the HAs examined are referred in Table 1. A detailed description and discussion of chemical and physicochemical properties of HAs examined can be found elsewhere (Loffredo and Senesi, 2005).

Briefly, the four HA samples show only some minor differences as a function of the horizon depth and/or the soil type. A larger N, S and OFR content and a slightly smaller aliphaticity characterize the surface horizon HAs, with respect to the deep horizon HAs. In particular, the much larger OFR concentration of both surface soil HAs than that of the corresponding deep soil HAs suggests a larger degree of polymerization and aromatic polycondensation for the former HAs with respect to the latter HAs (Senesi and Loffredo, 1999).

Further, a larger N content, E_4/E_6 ratio and aromatic character, and a slightly smaller C/N ratio, aliphaticity and carbonyl group content are measured for the P-HAs, with respect to the G-HAs. These differences, although limited, may be expected to affect differently the adsorption/desorption processes of HAs for EDCs.

Table 1. Some chemical properties of humic acids (HAs) isolated from soil samples used.

Parameter	P30 - HA	P90 - HA	G30 - HA	G90 - HA
C %	57.12	56.54	58.12	55.07
H %	6.19	5.35	5.80	6.44
N %	4.75	3.94	3.58	3.45
S %	0.41	0.19	0.43	0.37
O %	31.54	33.97	32.00	34.67
C/N atomic ratio	14.0	16.8	18.9	18.6
C/H atomic ratio	0.8	0.9	0.8	0.7
O/C atomic ratio	0.4	0.4	0.4	0.5
Carboxyl groups meq/g	2.63	3.28	3.10	3.22
Phenolic OH meq/g	4.28	3.55	2.63	4.88

Parameter	P30 - HA	P90 - HA	G30 - HA	G90 - HA
Total acidity meq/g	6.91	6.83	5.73	8.10
E4/E6 ratio	6.6	6.8	5.5	5.8
Organic free radicals spins/g x 1017	1.27	0.56	1.73	0.22

8.2 Adsorption Kinetics

Figure 4 shows two representative examples of adsorption kinetics curves of EDCS onto HAs. In any case, most adsorption (>90%) occurs rapidly in the first few hours of contact.

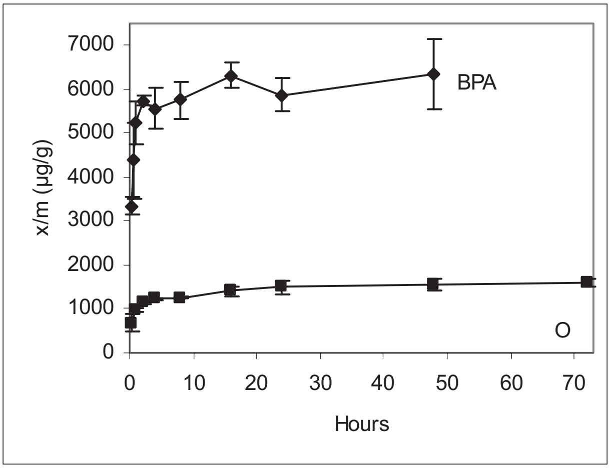

Figure 4. Adsorption kinetics of BPA and OP onto G-30-HA.

This phase is followed by a slow attainment of a steady-state equilibrium, which is reached generally in less than 24 h. BPA appears to be the most quickly adsorbed EDC. The rapid adsorption phase would occur on the most reactive and/or accessible sites of HAs, whereas the slower adsorption may reflect the involvement of less reactive and/or more sterically hindered sites.

8.3 Adsorption Isotherms

On the basis of the calculated correlation coefficients (r) values over the whole concentration range tested, experimental adsorption data of BPA and 17ED onto HAs fit best in a Langmuir model (Figs. 5 and 6). The related correlation coefficients, r, and the distribution coefficients, K_d, are referred in Table 2.

Table 2. Correlation coefficients, r, adsorption parameters, K_f and 1/n, and distribution coefficients, K_d, for endocrine disruptors adsorption onto soil humic acids (HAs).

Sample	r	K_f (L/Kg)	1/n	K_d (L/Kg)
		BPA (Langmuir)		
P30-HA	0.986	n.a.	n.a.	273
P90-HA	0.997	n.a.	n.a.	59
G30-HA	0.994	n.a.	n.a.	165
G90-HA	0.986	n.a.	n.a.	87
		17ED (Langmuir)		
P30-HA	0.985	n.a.	n.a.	1397
P90-HA	0.999	n.a.	n.a.	554
G30-HA	1.000	n.a.	n.a.	1405
G90-HA	0.995	n.a.	n.a.	933
		OP (Linear)		
P30-HA	0.988	1158	n.a.	1341
P90-HA	0.992	889	n.a.	905
G30-HA	0.992	1398	n.a.	2326
G90-HA	0.975	351	n.a.	503
		EED (Freundlich)		
P30-HA	0.958	1430	0.97	1586
P90-HA	0.990	780	0.95	828
G30-HA	0.994	1489	0.89	1778
G90-HA	0.961	852	1.03	889

n.a.: not applicable

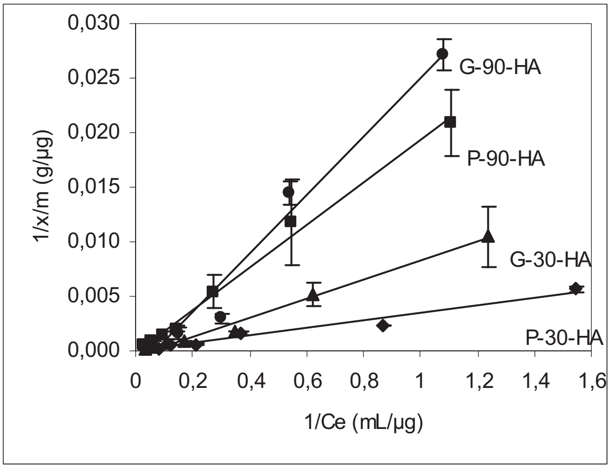

Figure 5. Langmuir adsorption isotherms of BPA onto soil humic acids (HA).

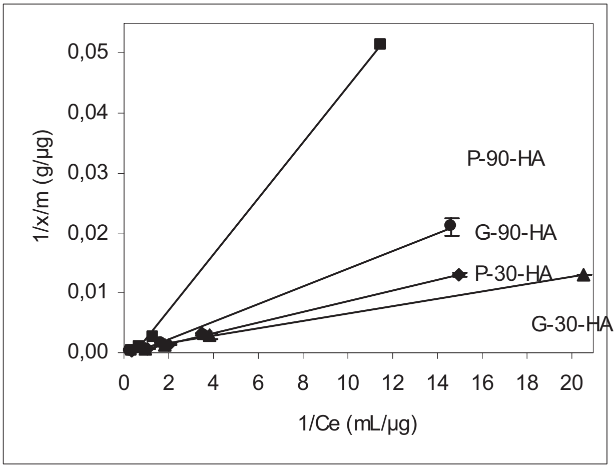

Figure 6. Langmuir adsorption isotherms of 17ED onto soil humic acids (HA).

A Langmuir isotherm is characterized by linear uptake at low concentrations transitioning to nonlinear (concave-down) uptake as sites are filled, finally leveling off at a maximum adsorption when the sites become saturated. The K_d value, i.e., the adsorption capacity of any HA for 17ED is much larger (about one order of magnitude) than that for BPA. Further, the trend of adsorption capacity of the various HAs for BPA and 17ED is almost similar, showing that surface soil HAs adsorb much more (about two to five times) EDC than deep soil HAs.

Experimental adsorption data of OP onto HAs fit best in a linear, C-type, adsorption isotherms (Figure 7). In all cases, no limiting adsorption (saturation) is observed for OP. The correlation coefficients, r, the Freundlich adsorption coefficient, K_f, and the distribution coefficients, K_d, are referred in Table 2. A linear, C-type isotherm indicates that a constant partition of OP occurs between the solution and the HA substrate, that is, adsorption is directly proportional to the solution concentration and no saturation occurs over the whole concentration range examined. Although slightly different, the K_f and K_d values follow a similar trend with values of surface soil HAs a little larger for P30-HA or much larger (about four times) for G30-HA than those of the corresponding deep soil HAs. Similar to 17 ED, OP is much more adsorbed than BPA.

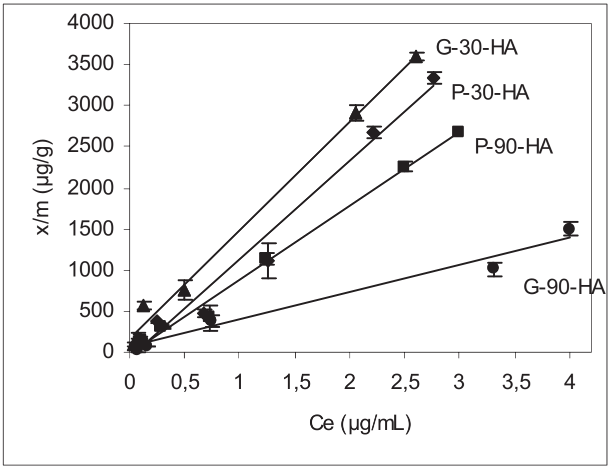

Figure 7. Linear adsorption isotherms of OP onto soil humic acids (HA).

Experimental adsorption data of EED onto HAs are best described by nonlinear, L-shaped (1/n<1) Freundlich isotherms (Figure 8). A nonlinear, L-shaped isotherm indicates that EED sorption occurs with increasing difficulty as adsorption sites are filled, but never reachs saturation up to the liquid-phase solubility. The correlation coefficients, r, the Freundlich adsorption parameters, K_f and 1/n, and the distribution coefficients, K_d, are referred in Table 2. Calculated values of 1/n are generally very close to unity

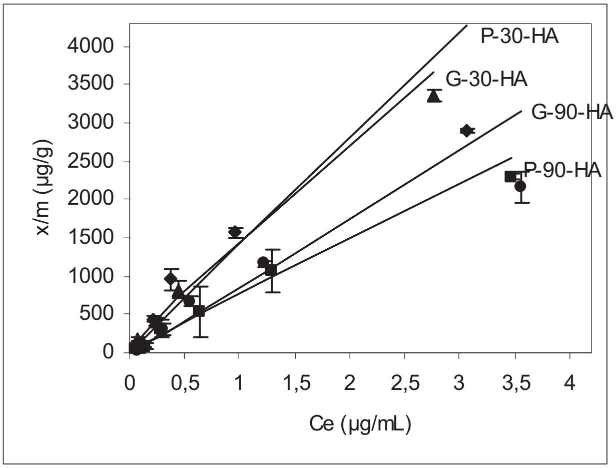

Figure 8. Freundlich non linear adsorption isotherms of EED onto soil humic acids (HA).

thus an almost linear, C-type adsorption model for EED can be considered also valid. Similar to OP, also the corresponding K_f and K_d values of EED are different but follow the same trend, with values of surface soil HAs about twice larger than those of the corresponding deep soil HAs. Similar to OP and 17ED, also EED is much more adsorbed than BPA.

8.4 Adsorption-Desorption Studies

Even if adsorption data of each EDC onto HAs could be best fitted in one of the three equations described above, a nonlinear Freundlich equation was used in all cases to fit both experimental adsorption data and desorption data, in order to obtain adsorption and desorption parameters comparable for any EDC and any substrate examined. This procedure is acceptable on the basis of the generally good correlation coefficients obtained for any EDC and any HA for a nonlinear Freundlich fitting of adsorption and desorption data.

Correlation coefficients, r, Freundlich nonlinear adsorption and desorption parameters, and total amounts (averages of three replicates) of EDCs that are desorbed during the experiment from each HA, expressed as the percentage of the initially adsorbed amount (100%), are shown in Table 3. The percentages of initially adsorbed (100%) EDC that remains adsorbed onto HA after each desorption step are shown graphically in Figs. 9, 10 and 11.

Table 3. Correlation coefficients, r, for Freundlich nonlinear isotherms, Freundlich adsorption and desorption parameters and total amount desorbed after a number of desorption steps indicated between parentheses, for endocrine disruptors onto soil humic acids (HAs).

Sample	***ADSORPTION***			***DESORPTION***			
	r	K_{ads} (L/Kg)	$1/n_{ads}$	r	K_{ads} (L/Kg)	$1/n_{ads}$	% desorbed
				BPA			
P30-HA	0.973	326	0.85	0.932	44.3	1.55	100 (4)
P90-HA	0.996	51	1.08	1.00	2.82	2.14	100 (2)
G30-HA	0.992	131	1.13	0.994	23.8	1.84	100 (3)
G90-HA	0.958	51	1.24	0.948	48.8	1.07	100 (3)
				OP			
P30-HA	0.980	1115	0.84	0.983	3028	0.08	26 (5)
P90-HA	0.979	857	0.98	0.995	2400	0.09	31 (5)
G30-HA	0.972	1662	0.80	0.980	3336	0.08	25 (5)
G90-HA	0.967	440	0.91	0.988	968	0.28	70 (5)
				EED			
P30-HA	0.958	1430	0.97	0.920	971	0.60	87.2 (6)
P90-HA	0.990	780	0.95	0.909	730	1.08	100 (4)
G30-HA	0.994	1489	0.89	0.984	1102	0.78	88.4 (6)
G90-HA	0.961	852	1.03	0.963	721	0.82	93.3 (6)

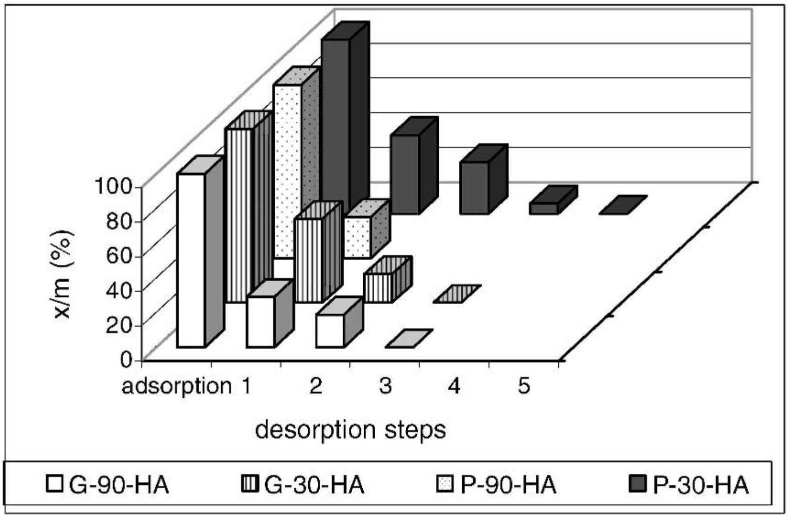

Figure 9. Percentages (%) of initially adsorbed (100%) BPA that remain adsorbed onto each humic acid (HA) after each desorption step.

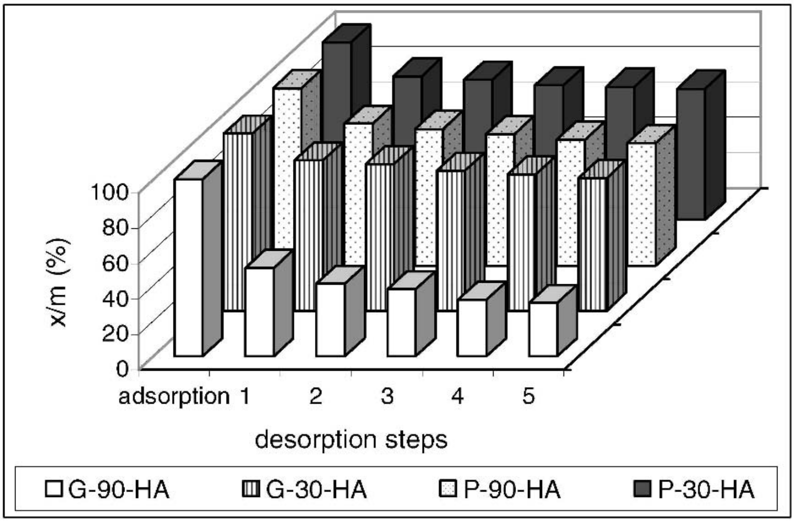

Figure 10. Percentages (%) of initially adsorbed (100%) OP that remain adsorbed onto each humic acid (HA) after each desorption step.

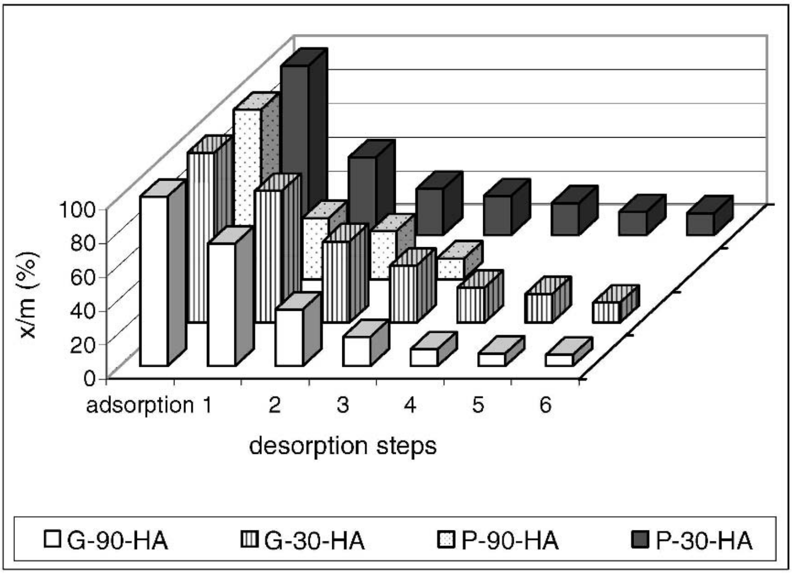

Figure 11. Percentages (%) of initially adsorbed (100%) EED that remain adsorbed onto each humic acid (HA) after each desorption step.

For all HAs the K_{des} values of BPA are smaller or much smaller than the corresponding K_{ads} values and, with the exception of G90-HA, the values of $1/n_{des}$ are larger than those of $1/n_{ads}$. These results suggest that a largely reversible adsorption of BPA occurs onto HAs. Desorption of BPA is generally completed (100%) after two to four desorption steps.

For all HAs the K_{des} values of OP are larger (up to three times) than the corresponding K_{ads} values, and $1/n_{des}$ values are much smaller (up to four orders of magnitude) than the corresponding $1/n_{ads}$ values. These results suggest for all HAs a marked irreversibility of OP adsorption. After five desorption steps, HAs desorb OP at various extents ranging from a maximum of 70% (G90-HA) to a minimum of 25% (G30-HA), thus complete desorption never occurs.

In the case of EED, all HAs show K_{des} values slightly smaller and $1/n_{des}$ values slightly smaller or slightly larger than the corresponding K_{ads} and $1/n_{ads}$ values, respectively. These results suggest the occurrence of a partially reversible adsorption. A total (100%) desorption of initially adsorbed EED occurs only for the sample P90-HA after six desorption steps, whereas the other HAs retain variable but small amounts of EED at the end of the experiment.

9. CONCLUSIONS

In soil, HS both in the solid and dissolved phases are the privileged components able to interact with AOPs in several ways, thus affecting the behavior, performance and fate of AOPs. In particular, adsorption processes directly or indirectly control all other processes that involve AOPs in soil, i.e., solubility and mobility, degradation and decomposition, etc. Rarely a unique process is involved in the interaction. More often, several processes with different mechanisms occur side by side, one or a few of which may dominate for a given AOP under given conditions. The type and extent of interaction may change with time and may ultimately result in the immobilization or reversible retention of the AOP which may result in a time-delayed risk of contamination. A better knowledge of the chemical nature and reactivity of HS and a better understanding of the mechanisms of their interactions with AOPs are expected to help in the progress of the description of AOP fate in soil. Advanced techniques that are available and show promise for the molecular and mechanistic investigation of HS-AOP interactions include Fourier transform infrared, nuclear magnetic resonance, electron spin resonance and fluorescence spectrocopies.

The soil HAs examined are slightly different one from another in their compositional, structural and functional properties, on dependence of soil origin and horizon. These differences, however, are shown to affect variously the adsorption/desorption processes of EDCS onto HAs.

Adsorption kinetics experiments show that the rate of adsorption of any EDC onto any HA is very fast (few hours) in the first phase which corresponds to more than 90% of total adsorption. A much slower phase follows which needs several hours until equilibrium. BPA appears to be the most rapidly adsorbed EDC onto all HAs.

Adsorption of BPA and 17ED is best described by a Langmuir model, which indicates that a saturation adsorption state is reached onto all substrates for these EDCs. Adsorption data of OP are best fitted in a linear, C-type isotherm, and those of EED in a Freundlich non-linear, L-shaped isotherm, thus indicating that no saturation occurs for adsorption of these EDCs onto any HA. The adsorption capacity of surface soil HAs for any EDC is greater (up to four times) than that of deep soil HAs. Further, the extent of adsorption onto HAs is generally smaller for BPA than for the other three EDCs that show a similar extent of adsorption onto HAs.

A comparison of adsorption and desorption data shows that: (a) with the exception of sample G90-HA, BPA adsorption is totally reversible with complete desorption occurring in few steps with a rate faster than that of adsorption; (b) OP adsorption is markedly irreversible with complete desorption never obtained and occurring at a rate much slower than that of adsorption; and (c) EED adsorption from most HAs is partially reversible, its rate of desorption is similar to that of adsorption, and a complete desorption is obtained only from sample P90-HA, whereas other HAs retain variable, generally low amounts of EED at the end of the experiment.

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INCORPORATING BIOAVAILABILITY INTO CRITERIA FOR METALS

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Abstract: Ecotoxicological effects of metals in aquatic and terrestrial environments often do not correlate well to the total concentration of metal. Environmental quality criteria and standards based on total concentration of a metal may over or under predict actual effects. Next to the physiology of the various species, a number of chemical environmental factors, particularly organic matter, pH, Ca, Mg and Na, affect the toxicity of metals such as Cu. To account for the modifying effect of these factors on the interaction of the metal with a biological receptor (called the biotic ligand) a chemical equilibrium model called the Biotic Ligand Model (BLM) has been developed to predict toxicity to aquatic organisms. This model considers competitive interactions of the metal, hydrogen and hardness ions, and natural organic matter in the aqueous phase and computes its speciation. The biotic ligand is modelled as an additional ligand in the system. Hydrogen, Ca, and Mg ions compete with the metal for the biotic ligand binding sites and thus affect metal binding to the organism and the ensuing toxicity. Toxicity is proportional to the fraction of the total biotic ligand sites occupied by the toxic metal. The BLM concept has been used for the development of both acute and chronic models with various aquatic species such as algae, invertebrates and fish. The Terrestrial Biotic Ligand Model (TBLM) is presently under development. Only soils for which soil organic matter is the principal binding phase for metal have been considered thus far. In the TBLM, the pore water speciation of the metal is predicted based on the metal content of the soil, soil organic matter, soil pH and the concentration of major ions in solution. Interaction of the pore water metal and modifying ions (H^+, Ca^{2+}, and Mg^{2+}) are handled in the same manner as in the aquatic BLM. These new models will permit the risk assessor to account for differences in metal bioavailability occurring in different

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regions and increase the environmental realism of the risk assessments of metals.

Key words: metals; bioavailability; speciation; Free Ion Activity Model (FIAM); Biotic Ligand Model (BLM); Terrestrial Biotic Ligand Model (TBLM); Windermere Humic Aqueous Model (WHAM)

1. INTRODUCTION

It has been frequently shown that the total concentration of a metal in an aquatic or a terrestrial ecosystem is poorly correlated to ecotoxicological effects. The toxicity of copper and other metals is profoundly affected by their physical and chemical form (Allen, 1993). Many studies that were conducted in the 1970's demonstrated the importance of considering metal speciation in evaluating ecotoxicological effects in the environment (see reviews by Hodson et al., 1979; Luoma, 1983; O'Donnel et al., 1985; Flemming and Trevors, 1989). Kelley (1988) wrote "...the speciation of a metal, rather than its total concentration, is the key to understanding its effect on the biota."

Because of the propensity of copper to form complexes with organic matter and the availability of electroanalytical measurement techniques that permit chemical forms to be differentiated, many studies have been conducted on its speciation.

Steemann Nielsen and Wium-Andersen (1970) recognized that free copper ions, at the level of copper found in natural waters, are very toxic to algae. They reported that the predominant forms of copper are not free copper ions, but are organic complexes and they reported these to be "not poisonous to algae". The understanding of the relationship between speciation and toxicity was greatly enhanced by the work of Sunda and Guillard (1976) who determined copper ion activity using a copper ion selective electrode and found that the algal growth rate was related to the free copper ion activity and not to the total copper concentration, which they had varied independently.

Some investigators have reported that chemical species other than free, ionic metals are toxic. This has been critically reviewed by Campbell (1995). Cowan et al. (1986) statistically analyzed available data in terms of the inorganic copper species that were toxic. Other investigators have found that not all organic copper species are non-toxic. Florence and Stauber (1986) and Florence et al. (1992) reported that lipid soluble copper(II) complexes are highly toxic to the marine diatom, *Nitzschia closterium*. Guy and Kean

(1980) and Borgmann and Ralph (1983) found that complexes of copper with synthetic ligands were toxic, but at reduced levels compared to free copper ion. Both Meador (1991) and Tubbing et al. (1994) reported that copper complexed with natural organic matter contributed to toxicity.

In summary, the numerous studies published over the past decades have clearly indicated that metal toxicity to aquatic organisms may be mitigated to by physico-chemical characteristics such as dissolved organic carbon (DOC), pH, water hardness, Ca, Mg, Na, alkalinity (see Paquin et al., 2002; Niyogi and Wood, 2004 for extensive reviews). The key question for regulators and applied environmental scientists is, however, how to incorporate all this fundamental, fragmentary and sometimes conflicting knowledge into a realistic and yet pragmatic way to include metal bioavailability into environmental regulatory frameworks.

2. WATER QUALITY CRITERIA

In the United States, Water Quality Criteria (WQC) have been established for the protection of aquatic life. It is desired that 95% of the species be protected. Because the toxicity cannot be measured for all species, a minimum data base is required. Appropriate statistics are employed to allow the criteria to be applied to species not tested. Most testing that is conducted is for assessing the acute toxicity, not for chronic toxicity. The WQC are based on an extrapolation from acute LC50 values to chronic values. National Water Quality Criteria are developed following the procedures detailed in the Guidelines document (Stephan et al., 1985).

As the toxicity of copper and a number of other metals has been found to depend on water hardness, this factor was incorporated into the criteria. The effect of other water quality factors, e.g. pH and the presence of DOC, that affect the toxicity are not considered in these criteria. Metal criteria are expressed in dissolved metal concentration (Prothro, 1993).

The manner in which the WQC are used is illustrated by the following example. The freshwater chronic criteria for a four-day average copper concentration (in µg/L) that is not to be exceeded more than once every three years on the average is given by the expression $e^{(0.8545[\ln(\mathrm{hardness})]-1.465)}$. The acute criteria value (in µg/L), based on a one-hour average concentration should not exceed the numerical value $e^{(0.9422[\ln(\mathrm{hardness})]-1.464)}$ more than once every three years on the average. For hardness of 50, 100 and 200 mg/L as $CaCO_3$, the four-day average concentrations of copper are 6.5, 12 and 21 µg/L, respectively, and the one-hour average concentrations are 9.2, 18, and 34 µg/L.

A single numerical value, even when modified by water hardness, does not provide the predictability of aquatic life effects that are desired for national criteria. Other factors, particularly pH and organic matter, modify the availability of copper and other metals to aquatic organisms (Erickson et al., 1996). Site-specific modification of the criteria is necessary for them to be predictive of effects at a specific location. In the USA, the Water Effects Ratio (WER) is a recommended means to provide site-specific modified criteria (Stephan et al., 1985; U.S. EPA, 1992, 1994; Prothro, 1993). To establish a WER, toxicity tests are conducted in a site water and in a reference or laboratory water. Reference water tests are used as surrogates for the laboratory tests that were used to derive national criteria. The ratio of the toxicities (WER) is used as a multiplier to adjust the National Water Quality Criteria (NWQC) to account for differences in bioavailability, as measured by toxicity tests, that would be applicable to that site. For example:

$$\begin{aligned}\text{Site - Specific WQC} &= \text{NWQC} \times \text{WER} \\ &= \text{NWQC} \times \frac{\text{Site Water LC50}}{\text{Reference Water LC50}}\end{aligned} \tag{1}$$

Allen and Hansen (1996) have discussed the importance of speciation in establishing the WER. Because the amount of complexation ability of a water sample is of the same magnitude as the amount of metal that must be added to produce the LC50, equation 1 implies that the WER for a given site will depend on the sensitivity of the organism being tested.

A major drawback of this approach is the need for performing ecotoxicity tests for every site considered. Additionally, financial constraints make that only acute tests with (mostly) one species can be used, which introduces considerable uncertainty when using the WER for protecting ‘all aquatic life’ at that particular site. Noteworthy is the fact that for copper site specific criteria can differ from NWQC by more than an order of magnitude illustrating the water-dependent bioavailability changes of copper.

3. BIOTIC LIGAND MODEL

The WER approach results in site specific criteria but it does not explicitly relate the criteria to specific chemical characteristics of the surface water and as such cannot predict what the effects of changes in these factors would have on toxicity. Research in two areas that was reported in the 1990s has allowed the development of models capable of predicting toxicity for fish, invertebrates and algae. The BLM provides a working mathematical model to predict toxicity of metal to aquatic organisms (Di Toro et al., 2001). It is based on the gill interaction model, which was proposed by Pagenkopf in 1983, and the free ion activity model of toxicity (FIAM), which has been reviewed by Morel (1983) and Campbell (1995). The BLM, which is shown schematically in Figure 1, accounts for metal complexation with natural organic matter (principally humic substances) and inorganic ligands, and the interaction of the free metal ion with critical receptor sites on organisms. The BLM also accounts for the interaction of alkaline earth metals, alkali metals and protons with humic substances and with the biotic ligand. It is easily seen in Figure 1 that the law of mass action would result in displacement of metal from humic substances due to an increase in calcium or protons. However, the calcium or protons would compete with the metal ions for the biotic ligand. These interactions lent themselves well to the development of the BLM as a computer code to simplify the computations.

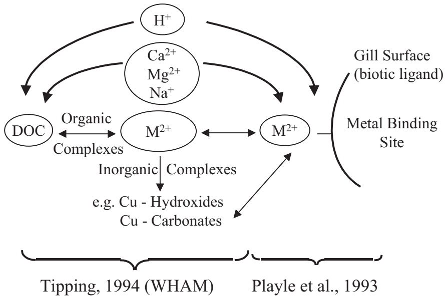

Figure 1. Schematic diagram of reactions considered in the Biotic Ligand Model (BLM) (adapted from Di Toro et al., 2001).

The chemistry of humic substances is complicated due to the fact that these materials are not discrete chemical substances but rather are complex mixtures. Several models have been developed that accurately describe the proton and metal binding to humic substances. The most widely used of these is Tipping's (1994) WHAM V, Windermere Humic Aqueous Model, which considers humic substances to be comprised of discrete sites that can exchange protons or metal ions.

Pagenkopf (1983) developed a chemical model to explain how changes in the hardness, alkalinity and pH values of the water affected metal toxicity to fish. Gill surfaces contain phospholipids that provide coordination sites for trace metals. The gill surfaces were treated as Lewis bases and the cations were treated as Lewis acids. The most important point of the model is that it allows the gill receptor sites to be modeled as ligands that react with metal ions in the water. The model predicts that increased hardness ion concentration will decrease metal uptake. Indeed, trace metal toxicity decreases in the presence of higher concentrations of calcium and magnesium. Organic ligands reduce the toxicity of toxic metals, but the degree of this reduction will depend on the relative affinity of the toxic metal for the ligand and for the gill surface.

Playle et al. (1993) determined the amount of metal associated with fish gills and the competitive effect of protons and calcium ions. From this they were able to compute the stability constants for the association of metals and protons with the gills. The extent to which Ca^{2+}, Mg^{2+}, Na^+, K^+ ions and pH independently mitigate acute copper toxicity for the cladoceran *Daphnia magna* was examined by De Schamphelaere and Janssen (2002).

The mass balance for the total biotic ligand site concentration is given by the equation

$$[\mathrm{BL\text{-}TOT}] = [\mathrm{CuBL}^+] + [\mathrm{CaBL}^+] + [\mathrm{MgBL}^+] + [\mathrm{NaBL}^0] + [\mathrm{HBL}^0] + [\mathrm{BL}^-] \tag{2}$$

in which the concentrations of the cation-biotic ligand complexes and of the unoccupied biotic ligand sites are expressed in mol/L. Equilibrium expressions for the binding of the cations can be written as conditional stability constants (L/mol) of the form

$$K_{\mathrm{CuBL}} = \frac{[\mathrm{CuBL}^+]}{\{\mathrm{Cu}^{2+}\}[\mathrm{BL}^-]} \tag{3}$$

in which the activity of the copper ion is in mol/L and the concentrations of the copper biotic ligand complex and the unoccupied biotic ligand sites are expressed in mol/L.

Substitution of equilibrium relationships of the type in Eq. 3 into the expression for [BL-TOT] in Eq. 2 leads to

$$\begin{aligned}[\mathrm{BL} - \mathrm{TOT}\} = [\mathrm{BL}^-] \Big(1 &+ K_{\mathrm{CuBL}}\{\mathrm{Cu}^{2+}\} + K_{\mathrm{CaBL}}[\mathrm{Ca}^{2+}] \\ &+ K_{\mathrm{MgBL}}[\mathrm{Mg}^{2+}] + K_{\mathrm{NaBL}}[\mathrm{Na}^+] + K_{\mathrm{HBL}}\{\mathrm{H}^+\}\Big)\end{aligned} \tag{4}$$

The BLM assumes that that the level of the observed effect is related to the fraction (*f*) of the total biotic ligand bound by Cu^{2+}

$$f = \frac{\left[\mathrm{CuBL}^{+}\right]}{\left[\mathrm{BL\text{ - }TOT}\right]}$$

$$= \frac{K_{\mathrm{CuBL}}\left\{\mathrm{Cu}^{2+}\right\}}{1 + K_{\mathrm{CuBL}}\left\{\mathrm{Cu}^{2+}\right\} + K_{\mathrm{CaBL}}\left[\mathrm{Ca}^{2+}\right] + K_{\mathrm{MgBL}}\left[\mathrm{Mg}^{2+}\right] + K_{\mathrm{NaBL}}\left[\mathrm{Na}^{+}\right] + K_{\mathrm{HBL}}\left\{\mathrm{H}^{+}\right\}} \tag{5}$$

The value of *f* is then used as the dose metric in the evaluation of toxicity rather than the total copper or free copper ion concentration. It is important to note that the fraction of the sites occupied by the metal is independent of the concentration of sites. This is true as long as the speciation of the copper shown in Figure 1 is unaffected by the introduction of the biotic ligand to the system. That is, the number of biotic ligand sites is small relative to the total metal concentration in the system. This is the normal situation for environmental systems.

The BLM requires a minimal amount of input data and the data that is required does not require sophisticated analytical instrumentation. The data input necessary are the concentrations of soluble metals (Cu or other metal whose toxicity is being investigated, Ca and Mg), the pH and concentration of dissolved organic carbon. The speciation is computed from these data.

The BLM has been used to predict toxicity for both laboratory and field waters. Figure 2 shows the toxicity testing results with fish exposed to copper in laboratory and field-collected water samples. The measured LC50 values range approximately 300-fold. The BLM is able to predict the result for most samples within a factor of 2.

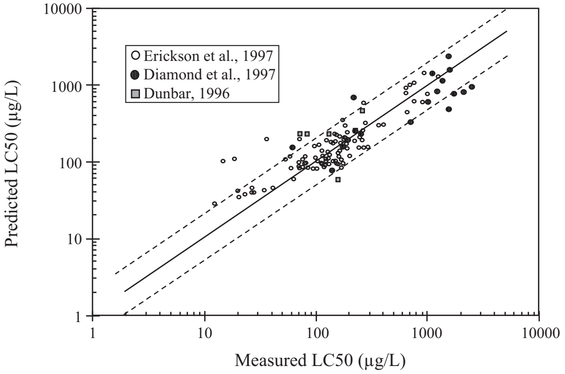

Figure 2. Comparison of Biotic Ligand Model (BLM) prediction of LC50 for Cu compared to measured values (modified from Santore et al., 2001). The 1:1 (solid) and 2:1 and 1:2 (dotted) reference lines are shown.

Initially, the BLM was developed, using existing literature datasets, for acute toxicity to fishes and then invertebrates, particularly daphnids. Recently, extensive research has led to the development of acute and chronic BLMs or similar approaches to predict metal toxicity to algae, invertebrates and fish (e.g. Bossuyt et al., 2004; De Schamphelaere and Janssen, 2002, 2004; De Schamphelaere et al., 2003, 2004, 2005 a, b; Heijerick et al., 2005; Villavicencio et al., 2005). Extensive validation of these models using surface waters collected throughout Europe has led to the acceptance of this science by EU regulators. Indeed, the chronic models developed with species of the above mentioned three trophic levels are currently being used or considered in the EU (regulatory) risk assessments of zinc, nickel and copper. In the USA, water quality criteria for copper based on the BLM are being proposed by the U.S. Environmental Protection Agency (U.S. EPA, 1999).

4. TERRESTRIAL BIOTIC LIGAND MODEL

In terrestrial systems there is the same basic problem with adequacy of criteria that was present in aquatic assessments prior to the development of the aquatic BLM. Also for the terrestrial compartment one needs to account for the differences in soil properties to be able to account for differences in ecotoxicological response of soil. Again, understanding the main mechanisms which are responsible for these differences means that one is to predict the ecotoxicological response rather than just to explain it. Finally, when developing such a predictive model, it should require minimal data input and the data should be of a routine nature that can be obtained by most laboratories.

It has been demonstrated that a BLM approach is applicable to predicting effects of metals to soil organisms (Van Gestel and Koolhaas, 2004; Hough et al., 2005; Steenbergen et al., 2005). In these studies metal in soil pore water was the starting point for the assessment. Like the aquatic BLM, they demonstrated that the effects could be accounted for by considering the activity of the free metal ions with competition at the receptor site.

Plette et al. (1999) presented a model that coupled metal partitioning to the soil with the toxicity of the metal ion in soil solution. Because the results were based on literature reports and not on an integrated program of soil chemistry and ecotoxicology measurement, only a qualitative analysis was possible.

Thakali et al. (2005) have developed a Terrestrial Biotic Ligand Model (TBLM) which is shown schematically in Figure 3. This was applied to a series of soils that had been spiked with copper or nickel and tested with the barley root elongation ecotoxicity assay. The version of the TBLM that they presented considers only non-calcareous soils. In non-calcareous soils with soil organic matter greater than a few tenths of a percent, the only phase in the soil that significantly binds copper is the organic matter. Indeed, Ponizovsky et al. (2006) demonstrated that in soils at or below field moisture capacity, the mass of metal in soil solution is insignificant relative to that sorbed to the soil organic matter. For this low moisture condition the free metal ion concentration in the soil solution is not dependent on the pore water composition. They used WHAM Model VI - with inputs of the total amount of metal added to the soil, soil pH and soil organic matter content - to predict the concentration of free copper ion in the soil pore water.

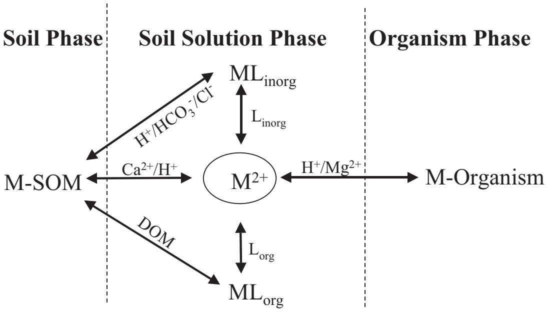

Figure 3. Schematic diagram of the reactions considered in the Terrestrial Biotic Ligand Model (TBLM) (based on Allen, 2001). Soil organic matter (SOM) is represented as the partitioning phase for metal in soil.

Interaction of the metal in the soil pore water with the biotic ligand is treated in the same manner as was discussed previously for aquatic toxicity. In the case of the effect of copper on barley root elongation, protons were found to be the only ions that significantly compete for the biotic ligand. The measured and TBLM-predicted barley root elongation shown in Figure 4 indicates that the TBLM predicted values compare well with the observed values. As such it can be concluded that this (preliminary) model sufficiently explains differences in metal bioavailability across soils to be useful for risk assessment purposes. Testing has now also been conducted to determine the effects of both copper and nickel on plant, invertebrate and microbial endpoints.

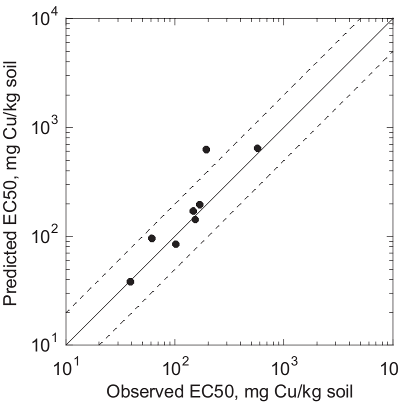

Figure 4. Comparison of Terrestrial Biotic Ligand Model (TBLM) prediction of the effect of copper dose to barley root elongation (RE) compared to measured values (modified from Thakali et al., 2005). The 1:1 (solid) and 2:1 and 1:2 (dotted) reference lines are shown.

5. CONCLUSIONS AND RECOMMENDATIONS

The BLM and TBLM approach provide a means to predict the ecotoxicological effect of metals on the environment. Their data requirements are small and only routine measurement are needed as model inputs. These models will be useful in projecting environmental response to management decisions including effects of modification of land use, remedial activities and long term sustainability.

Additional efforts are needed to develop and validate the chronic BLM for a number of metals for a range of sensitive organisms. The development of the TBLM is in its infancy. Validation experiments are needed. A sufficient number of endpoints should be investigated so that a species sensitivity distribution can be established. This should also be done for a number of metals.

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THE METAL UPTAKE AND ACCUMULATION IN FISH LIVING IN POLLUTED WATERS

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Abstract: Fish living in polluted waters tend to accumulate heavy metals in their tissues. Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits). Various metals show different affinity to fish tissues. Most of them accumulate mainly in liver, kidney and gills. Fish muscles, comparing to the other tissues, usually contain the lowest levels of metals. Metal distribution in various organs is time-related. Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances.

Key words: fish; metal; accumulation

The results of many field studies of metal accumulation in fish living in polluted waters show that considerable amounts of various metals may be deposited in fish tissues without causing mortality. Various metals are accumulated in fish body in different amounts. These differences result from different affinity of metals to fish tissues, different uptake, deposition and excretion rates. Metal levels in live fish usually follow the ranking: Fe > Zn > Pb > Cu > Cd > Hg. The levels of Zn may be very high, up to over 300 μg/g d. w. The maximum concentrations of lead and copper are lower and usually do not exceed 10 μg/g d. w. Cadmium and mercury are accumulated by the fish in very low amounts, below 1 μg/g d.w. Metal accumulation in fish depends on pollution, and may differ for various fish species living in the same water body (for a review see Jezierska and Witeska, 2001).

Generally, the higher metal concentration in the environment, the more may be taken up and accumulated by fish. Relationship between metal concentrations in fish and in the water was observed in both, field and laboratory studies (Moiseenko et al., 1995; Linde et al., 1996; Yamazaki

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et al., 1996; Zhou et al., 1998). It should be, however, emphasized that body metal level is related to its waterborne concentration only if metal is taken up by the fish from water. If food is main source of metal, such a relationship does not necessarily occur.

Metals in natural waters occur in particulate or soluble form. Soluble species include labile and non-labile fractions. The labile metal compounds are the most dangerous to fish. They include various ionic forms of different availability to fish. Many data show that the amounts of metals in the labile fraction, and the share of various metal ions strongly depend on environmental conditions.

The environmental factors affect the uptake and accumulation of metals in fish. According to Kock et al. (1996), cadmium and lead levels in *Salvelinus alpinus* liver and kidneys indicate higher uptake rates of both metals in summer when water temperature was higher. The authors explained that with increased metabolic rate. The data obtained by Douben (1989) indicate that the rate of uptake and elimination of cadmium by *Noemacheilus barbatulus* increased with water temperature, the author suggested stronger effect of temperature on metal absorption than on elimination.

Water temperature may cause the differences in metal deposition in various organs. Higher temperatures promote accumulation of cadmium especially in the most burdened organs: kidneys and liver (Yang and Chen, 1996). Increased accumulation of metals by fish at higher temperatures probably results from higher metabolic rate, including higher rate of metal uptake and binding.

Many data indicate that water acidification directly affects metal accumulation rates by the fish. Comparison of the data concerning metal levels in fish from various lakes indicates that the concentrations of cadmium and lead, but not zinc, are considerably higher in the fish from acidified lakes (Grieb et al., 1990; Haines and Brumbaugh, 1994; Wiener et al., 1990; Horwitz et al., 1995). Accumulation of copper is also higher at lower pH (Cogun and Kargin 2004). Summarizing, we may conclude that water acidification affects bioaccumulation of metals by the fish in an indirect way, by changing solubility of metal compounds or directly, due to damage of epithelia which become more permeable to metals, and on the other hand, competitive uptake of H^+ ions may inhibit metal absorption.

Water hardness (mainly calcium concentration) considerably affects uptake of metals across the gill epithelium. According to Playle et al. (1992), enrichment of water with calcium reduced copper accumulation in the gills. Baldisserotto et al. (2005) reported that elevated dietary Ca^{2+} protected against both, dietary and waterborne Cd uptake. The results obtained by Barron and Albeke (2000) indicate that calcium reduces zinc uptake by

Oncorhynchus mykiss. Pagenkopf (1983) explained that calcium might compete with other metals for binding sites on the gill surface.

Similarly as hardness, also salinity reduces uptake and accumulation of metals by the fish. According to Stagg and Shuttleworth (1982), seawater-adapted *Platichthys flessus* showed lower copper concentration than freshwater-adapted individuals. The rate of lead accumulation by *Gillichthys mirabilis* was inversely proportional to the salinity of the medium (Somero et al., 1977). Natural waters are usually contaminated with mixtures of metals and other toxic compounds. Accumulation of certain metals in fish may be altered in the presence of the others (Wicklund et al., 1988; Cuvin-Aralar and Furness, 1990; Allen, 1994, 1995; Pelgrom et al., 1995). Interactions among various metals may be related to their different affinities to various organs. The results of various studies usually show synergistic or additive effects of metals but in some mixtures antagonistic effects may occur (for a review see Jezierska and Witeska, 2001).

Various species of fish from the same water body may accumulate different amounts of metals. Interspecies differences in metal accumulation may be related to living and feeding habits. Kidwell et al. (1995) observed that predatory fish species accumulated more mercury but the benthivores contained more cadmium and zinc. Higher concentrations of mercury in the predatory fishes comparing to the non-predatory ones was also reported by Voigt (2004). Ney and Van Hassel (1983) found that lead and zinc concentrations were higher in benthic fish. The results obtained by Campbell (1994) indicate that predators accumulated more zinc and nickel than benthivores, while the latter contained more cadmium.

In fish, concentrations of most metals (except for mercury) are usually inversely related to the age and size. Measurements of bioaccumulation of iron, manganese, zinc, copper, nickel and lead by *Pseudocrenilabrus philander* from a mine-polluted impoundment revealed that there was an inverse relationship between metal concentrations and body mass of fish (De Wet et al. (1994). According to Allen-Gill and Martynov (1995), an inverse correlation occurred between the age and lead content in *Coregonus clupeaformis*, and a similar relationship was found between accumulation of zinc, lead, cadmium and nickel and age of *Catostomus commersoni* (Ney and Van Hassel, 1983). The youngest fish showed the highest concentrations of metals, most distinct differences occurred for zinc. Negative relationships between fish length and metal concentrations (for Cr, Pb, and Cu) were also reported by Canli and Atli (2003). Field data show that mercury concentration in fish increases with age and size. Increase in body mercury level with fish age and size is probably related to the affinity of this metal to the muscle tissue (Goldstein et al., 1996; Munn and Short, 1997; Green and Knutzen, 2003; Voigt, 2004).

The data obtained by many authors indicate that metals show different affinity to various organs. The major part of total body loads accumulated at different concentrations of metals in the water, and at various exposure times are found in liver, kidney and gills. Some authors observed also considerable concentrations of metals in the digestive tract of fish from natural water bodies (Al-Mohanna, 1994; Kock et al., 1998; Giguere et al., 2004). High metal concentrations in the digestive tract of fish are related to the dietary uptake route. According to Yamazaki et al. (1996), the gall bladder of *Carassius auratus* contained high levels of such metals as silver, chromium and copper.

Such organs as the gonads, bones, and brain may also show high metal levels, while the muscles, comparing to the other tissues, usually show low concentrations of metals but are often examined for metal content due to their use for human consumption.

The affinity of various metals to fish organs may differ. Particularly, accumulation of essential metals such as iron, zinc, copper, manganase or cobalt is organ-specific. For example, even at low environmental concentrations, copper shows distinct affinity to the liver**,** while zinc concentrates in the gonads because in these organs they play their main metabolic roles. On the other hand, under conditions of metal contamination, metals tend to deposit in the same organs where they may exert toxic effects.

Cadmium is accumulated primarily in the kidney and liver, but it may reach high concentrations also in the gill, digestive tract and spleen. Lead deposits in various organs: liver, kidneys and spleen, but also digestive tract and gills. High levels of this metal are sometimes found in bone. The highest concentrations of zinc are often observed in the gills, but the digestive tract, liver and kidney may be also considerably burdened.

The accumulation of metal in fish in sublethal exposure is time-dependent. Usually, in the initial period of exposure metal is absorbed and accumulated at a high rate, then the level stabilizes when an equilibrium of metal uptake and excretion rates is attained.

Metal distribution in various organs is also time-related. Accumulation of metals in the organs of fish is a function of uptake and elimination rates, and metal concentrations in various organs may change during and after exposure, according to various patterns. The effect of time on metal distribution within the organism is a complex issue due to different affinity of various metals to the tissues of various fish species.

Many data indicate that dynamics of metal concentrations in various organs during exposure and depuration may be different (Fig. 1). At the beginning of waterborne exposure metal concentrations in the gills rapidly increase, and then usually decline. After the end of exposure metals are rapidly removed from the gills. In case of dietary exposure, metal levels in the gills increase much slower and usually reach lower values.

During dietary administration of metals, their concentrations in the digestive tract increase and remain high until the end of exposure, and rapidly decrease during depuration. In case of waterborne exposure, metal levels in the digestive tract are usually low.

Liver accumulates high concentrations of metals, irrespectively of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment. That is especially true for copper and cadmium. Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared.

Metal concentrations in the kidneys rise slower than in liver, and usually reach slightly lower values, except for such metals as cadmium and zinc that show very high affinity to kidneys, therefore the kidneys may be considered a good indicator of pollution too. During depuration, kidney metal levels remain high or may even increase for some time, which is related to the role of kidneys as excretory organs.

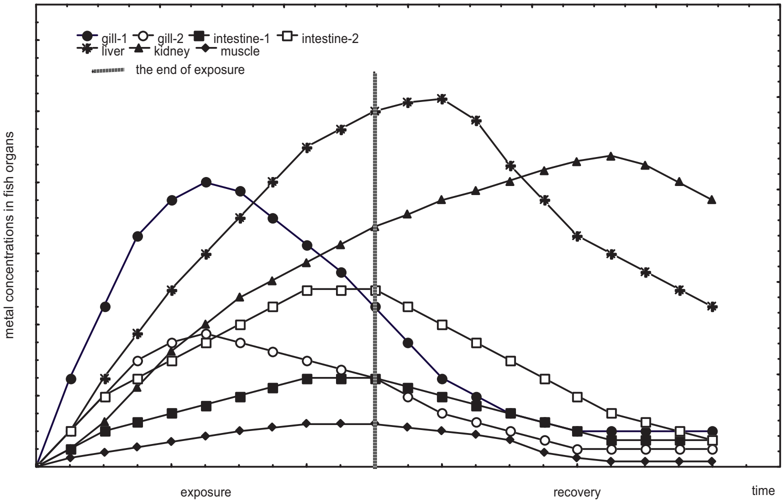

Figure 1. Metal distribution and dynamics in various organs of fish during and after exposure, 1 – waterborne exposure, 2 – dietary exposure (according to Jezierska and Witeska 2001).

Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances (for a review see Jezierska and Witeska, 2001). Adverse effects of metals on fish are related not only to material accumulation, but also to cumulative toxic effect. Exceeding certain values of metal concentration in fish results in lethal disturbances.

In most cases, fish from metal-contaminated water are safe for human consumption due to low metal accumulation (except for mercury) in the muscle tissue. However, such fish may constitute a potential risk for predatory fishes, birds and mammals feeding on contaminated fish.

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SENSORISTIC APPROACH TO BIOLOGICAL DAMAGE AND RISK ASSESSMENT

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Abstract: A review is proposed on various types of sensoristic approaches to biological damage and risk assessment. The traditional analytical approach to toxicity evaluation of a matrix is replaced by a sensor based one. Some sensor models are so presented able to offer an opportune solution to the problem to be solved. Some sensors are able to detect risky and dangerous situations and to evaluate them by measuring integral indexes such as total organic carbon, ecopermanence, total radical concentration, integral toxicity. Finally also a sensor based on human tissue is presented as example of a new generation biosensor.

Key words: TOC analysis; ecopermanence; TiO_2 photosensor; radicals biosensor; biosensor for integral toxicity assessment; biosensor based on human tissue

1. INTRODUCTION

The continuously increasing number of compounds, new and already unknown ones, disposed into the environment and often from environment to food chain obliges use of very complicated and time wasting controls and analysis in order to establish if alarm and risk situations for citizens and users are by chance reached. These analysis are generally very costly and so not always – and anyway not as frequently as needed – and not everywhere permitted. It is also to be observed that sometime the fear for the pollution of the considered matrix can produce wrong results, as pollution shows to be insignificant and below danger levels, so that the analysis could have been avoided, but obviously this cannot be foreseen.

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So a new approach must be probably more opportunely followed: the approach of the crossing light, preliminary screening and markers. The first step of this approach is a simple test on waiting for a red or a green light that means a negative or a positive sample: if green, detailed and specific analysis can be avoided, if red they must be carefully performed in order to interpret the negative indication. These analyses when performed can be anyway carried out with a preliminary screening able to concentrate the analysis on samples characterized by value of the key indexes near to the limit values (± 30% can be considered a reasonable range). Finally when the screening step obliges to deeper analysis, markers can be adopted able to give information and to classify the positive samples in an order of toxicity. The choice of these markers depends on the kind of matrix but it must be also dynamically considered as the research advancements continuously allow individual new markers able to be adopted for the fundamental role of integral parameters. Finally, when this marker test also indicates toxic levels, a complete full analysis generally by hyphenated methods such as GC - MS, HPLC - MS, CE - MS, LCP - MS has to be performed.

In this paper we want to contribute on proposing new instruments for the first steps of the proposed approach. Some sensors able to detect risky and dangerous situations (red crossing light) and to evaluate them by screening and marker procedure are presented. The markers we propose are total organic carbon, ecopermanence, total radical concentration, and integral toxicity.

2. SENSORS FOR A PRELIMINARY SCREENING

2.1 TiO_2 Sensor for TOC (Total Organic Carbon) Analysis

TOC is an instrumental measurement consisting of catalytic oxidation of total organic carbon to CO_2, the produced amount of which is assumed to evaluate TOC index. TOC is a primary marker of the healthy or unhealthy state of a river and a very used datum to monitor organic pollutants, both natural and synthetic. Luckily, most of the former are not noxious to humans, but the latter are on the contrary generally heavily involved in problems of safety and hygiene. Generally, the laws, national or international, do not give any limit value due to the fact that the same TOC values have different meaning according to the compounds accounting for them. So more relevant in many cases is the profile within the time: sharp up variations are surely to be feared and contrasted. The industrial analysers are based on the combustion at high temperature or on the couplement between an UV reactor and persulphate treatment with conductimetric or infrared determination of produced carbon dioxide.

There is an increasing demand for measurement of organic carbon in solutions both in industry and environmental research for the purpose of continuous water-quality monitoring. Practically all the methods used are based on a catalytic system in which metal oxides play a major role. The new TOC measurement system uses a mixed anatase-rutile form of TiO_2 as catalyst for the mineralization process, and direct measurement of the CO_2 produced, by a gaseous diffusion electrode. The entire research is based on heterogeneous catalysis using an immobilised catalyst, which can offer considerable advantage over other methods of catalysis. Four different catalytic systems were analysed. Three involved thermal immobilisation of TiO_2 on glass supports (glass spheres and glassy particulates) or on an Al metal grid. One further system consisted of direct oxidation of a Ti grid at high temperatures. The system was illuminated using a 350 nm UV source (350 mW/cm^2) contained in a home-made measurement cell. Five compounds, malic acid, pentachlorophenol, sodium dodecylsulphate, hydroquinone, and citric acid, which were deemed to be representative and had been investigated in several previous studies, were used as photodegradation targets. Chemometric analysis, by principal components analysis, allows the used catalytic systems to be easily compared each other and a single PC component is able to perform classification (Campanella et al., 2003, 2002a).

2.2 TiO_2 Photosensor of Ecopermanence

This is a preliminary approach to the use of a new generation of solid state sensors based on the capacity of the sensor element to catalyze the photodegradation of various kinds of organic compounds, to monitor the consequent pH variation and to recognise their structure on the basis of the type of process catalyzed. The electron holes present in the TiO_2 structure are able to trigger an oxidative process involving substances present in the environment, in particular those ones that can be adsorbed on it (Byrne et al., 2002; Dhananjeyan et al., 1997; Doong et al., 2001; Dvoranová et al., 2002; Epling and Lin, 2002; Fujishima et al., 1993; Maurino et al., 1997; Mishima et al., 1998; Oliva et al., 2002; Skubal et al., 2002; Vamathevan et al., 2002). These characteristics make titanium dioxide a suitable material for use as a sensor for antioxidant, i.e. reducing, substances and as a sensor for measuring environmental permanency and consequent risks.

By reacting with titanium dioxide antioxidant substances produce potential variations corresponding to values specific of the above mentioned photo-oxidative processes. In this first attempt, TiO_2 in the prevailing anatase form was used as sensor, and phenols as known compounds. The overall profile of the response, curve E = f(t), is diagnostic for the purpose of compound recognition, the characterisation of its state purity. The behaviour of nanoparticulate TiO_2 was tested in the presence of antioxidants (hydroquinone, ascorbic acid, n-propylgallate).

During mineralization acidification occurs (Fujishima et al., 1993). TiO_2 being a photocatalyst of the process, but also behaving as pH sensor, is so in the lucky position to activate a process and to monitor its proceeding. The time needed in order to record a pH shift to more acid values can be assumed as a delay time proportional to the recalcitrancy of the compound (Figure l). The principle was applied to some common pollutants obtaining the enclosed delay time value (Table 1). These values are in agreement with other molecular properties related to the stability of the considered compounds.

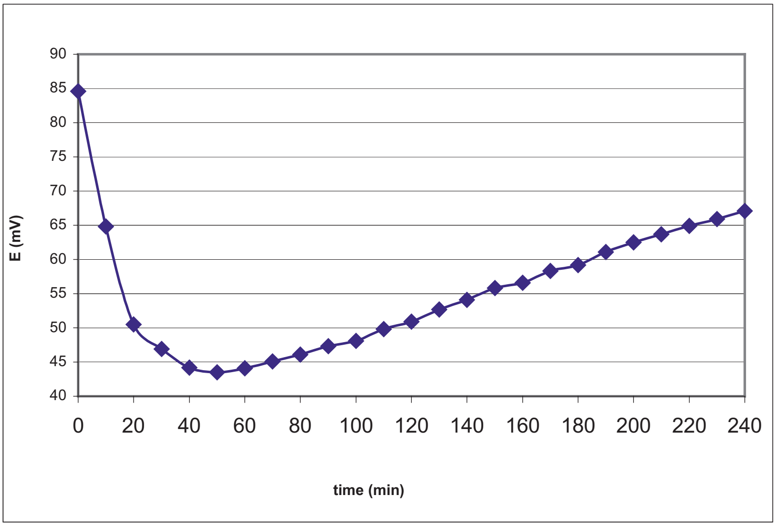


Figure 1. Curve E = f(t), E = titanium dioxide potential; t = irradiation time. The curve is diagnostic for the purpose of compound recognition The time needed in order to record a pH shift to more acid values (or a potential shift to increasing values) can be assumed as a delay time proportional to the recalcitrance of the compound.

Further information was obtained by the slope of the pH decrease following the beginning of the mineralization process indicated by the acidification after the delay time. The obtained values are shown in the last column of Table 1.

It can be tentatively proposed that the ratio between the values of the last column and the corresponding of the second column be considered as a parameter accounting for the environmental permanency or ecopermanence of a compound that we can reasonably assume as being inversely proportional to mineralizability.

Table 1. Delay time and slope of pH decrease values of some common pollutants.

Compound (10^{-2} mol/L)	Delay Time (min) (RSD% = ±6)	Slope (ΔpH/Δmin)
p-chlorophenol	30	0.16
m- chlorophenol	30	0.31
o- chlorophenol	60	0.22
hydroquinone	50	0.08
p-quinone	20	0.04
glucose	60	0.04
carbaryl	50	0.12

This parameter could be of great help in the case of unknown or uncharacterised compounds: a White Book of the European Community invites scientific community to make the most of efforts in order to set up chemical tests able to give information - especially alarm advises - in real - or almost real - time about the toxicity of a compound.

Several experimental details were thoroughly studied and optimised: they include nanoparticulate titanium dioxide in suspension, the presence in the water used as solvent of carbonate and organic substances (which affect sensor response), the buffering properties of phosphate over the entire linear range of detector response, the verification using alternative techniques of the results obtained, the rigorous analysis of the data (kinetic curves, uncertainty and equation describing the phenomenon); lastly, the size (owing to the growing demand for 'portable' instruments), ease of use and the materials characterising the sensor.

Titanium dioxide has also found profitable use as component of an electronic nose or tongue: by different types of treatments useful for the sensing film preparation different chemical species are able to be determined. The fingerprint so obtained can be interpreted as diagnostic on the basis of chemometric treatment of data.

2.3 Biosensor for Radicals

To the question about the contribution of chemistry to the evaluation of the human and environmental risk from chemicals and production, also a radical approach answer can be given. When we produce or use energy some of the energy remains segregated within secondary pathways molecules called radicals, which are highly energetic and reactive due to the presence in their electronic structure of unpaired electrons, and which can be so or lost or coupled. As a consequence, the radicals act as reducing or oxidising agents. Radicals are able to attack any substrate or tissue so behaving as very dangerous chemical species.

When a free radical reacts with a non-radical, a new radical results and chain reactions can be set up:

1. A radical ($X^{\bullet}$) may add on to another molecule. The adduct must still have an unpaired electron

$$X^{\bullet} + Y \rightarrow [X\text{-}Y]^{\bullet}$$

Example: $OH^{\bullet}$ adds to guanine in DNA; the initial product is an 8-hydroxyguanine radical

2. A radical may be a reducing agent, donating a single electron to a non-radical. The recipient then has an unpaired electron

$$X^{\bullet} + Y \rightarrow X^{+} + Y^{\bullet -}$$

Example: $CO_2^{\bullet -}$ reduces Cu^{+} to Cu

$$CO_2^{\bullet -} + Cu^{+} \rightarrow CO_2 + Cu$$

3. A radical may be an oxidizing agent, accepting a single electron from a non-radical. The non-radical must then have an unpaired electron left behind.

Example: Hydroxyl radical oxidises the sedative drug promethazine to the radical cation

$$PR + OH^{\bullet} \rightarrow PR^{\bullet +} + OH^{-}$$

4. A radical may abstract a hydrogen atom from a C-H bond. As the hydrogen atom has only one electron, an unpaired electron must be left on the carbon.

Example: Hydroxyl radical abstracts hydrogen from a hydrocarbon side-chain of a fatty acid to initiate lipid peroxidation.

$$CH + OH^{\bullet} \rightarrow C^{\bullet} + H_2O$$

Radicals particularly attack proteins and produce oxidative stress. All amino acid residues of proteins are potential targets for attack by reactive oxygen species (ROS) produced in the radiolysis of water; however, in only a few cases have the oxidation products been fully characterised. Moreover, under most physiological conditions, cysteine, methionine, arginine, lysine, proline, histidine and the aromatic amino acids are primary targets for ROS-mediated oxidation.

Exposure of proteins to reactive oxygen species can lead to the formation of protein-protein cross-linkages

$$2PSH + "O" \rightarrow P\text{-}S\text{-}S\text{-}P + H_2O$$

$$PNH_2 + PCHO^- \rightarrow PN{=}CHP + H_2O$$

$$2PNH_2 + CH_2(CHO)_2 \rightarrow PN{=}CHCH_2CH{=}NP + 2H_2O$$

$$2PCHR \rightarrow PCHR\text{-}PCHR$$

Moreover, radicals are present in the environment and their concentration, for instance in Roman urban air, monitored by us by means of opportunely designed and built sensors, is proportional to traffic intensity and the number of inhabitants of the considered area.

Our research group recently approached the problem of radical determination starting from the determination of oxygen free radicals, in particular superoxide radical, and assembling several new kinds of electrochemical sensors and biosensors suitable for this purpose (Campanella et al., 2001; 2000; 1999; 1997); firstly a voltammetric system based on the detection of reduced cytochrome c; this system was applied to develop a suitable amperometric carbon paste electrode; secondly two potentiometric sensors (one classical selective membrane sensor and the other a solid state field effect transistor sensor) based on selective membrane entrapping benzylidenephenylnitrone with potentiometric detection. More recently we studied two different kinds of biosensors to determine superoxide radicals obtained by coupling a transducer consisting of an amperometric gas diffusion electrode for the oxygen, or another amperometric electrode for hydrogen peroxide, with superoxide dismutase enzyme immobilized in kappa carrageenan gel. Both the sensors showed suitable response to the superoxide radical. We consider that the second type of biosensor is now mature both from an engineering and an operative point of view and employed this biosensor to check antioxidant properties of several compounds comparing the response of the biosensor both in presence and in absence of the considered scavenger compound.

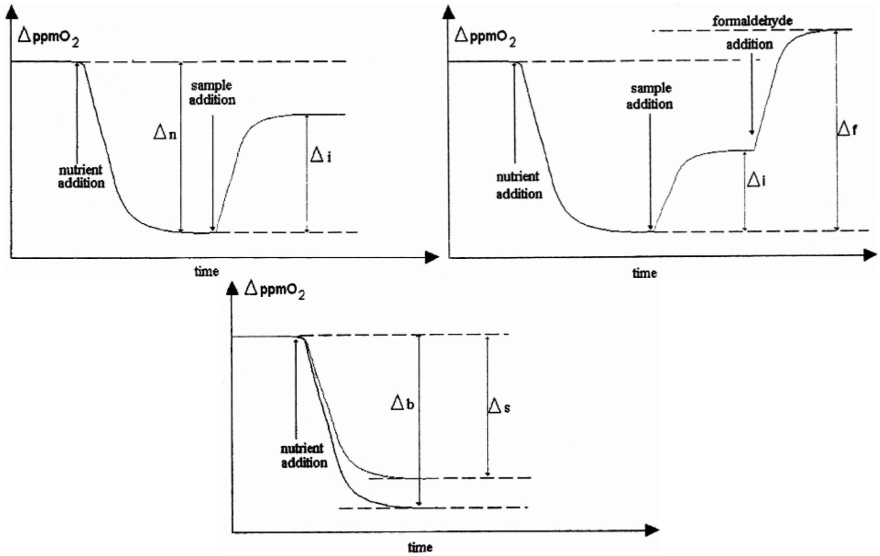
Using the SOD/H_2O_2 biosensor set up in our laboratory (Campanella et al., 1999; 1997), we recently evaluated the scavenging properties in vitro of important molecules such as cysteine, melatonin, β-carotene and acetyl salicylic acid able to protect humans from environmental damage.

2.4 Biosensor for Integral Toxicity Assessment

For some years now, the toxicity of many chemical substances has been studied by means of living organisms used as biological indicators. Higher organisms are generally used, such as the guinea pig, as the results they produce are often extremely reliable, even though the response times may be lengthy (ranging from a few days to several months, or even years in the testing for chronic toxicity). Nevertheless, by exploiting unicellular organisms, in particular yeasts, it is possible to reduce testing time considerably. Furthermore, by using suitable biological systems, such as immobilised yeast colonies, it is possible to implement the method even in-situ, with the advantage of obtaining a value of integral toxicity due neither to this nor to that compound but to all compounds together with the eventual antagonisms and synergisms included too.

An immobilised yeast cell biosensor has been developed for the total toxicity testing of a sample that may contain a number of different pollutant species; the biosensor uses an amperometric gas diffusion oxygen as indicator electrode (Campanella et al., 2002b). The method is based on the perturbation of the respiratory activity of a yeast, *Saccharomyces cerevisiae*, immobilised on an agar gel containing the culture medium (i.e., 'agarised medium') by the toxic test substance. Glucose is used as substrate while the substances tested consist of several metallic ions, phenols and cationic or anionic surfactants, pesticides and other toxics.

The measurements are performed by allowing the biosensor to stabilize, under magnetic stirring, in the glass cell thermostated at 25° C containing a fixed electrolytic solution (15 ml), isotonic with the yeast cell plasma. The next step is to add a standard glucose solution (final concentration 0.1% w/v), and the variation of dissolved oxygen concentration caused by the increased in the yeast cell respiration process recorded as a function of time; in practice, we obtained what is commonly called the 'respirometric curve', that shows the behaviour of the oxygen consumption as a function of the time: in fact it is well known that the metabolic map of the yeast cells, involving the glycolysis of glucose to pyruvate, is in connection with the ethanol production in the tricarboxylic acids cycle, linked to respiration process (Figure 2).



Procedure 1
Fill the measurement cell with the medium containing the yeast cells and stir
Dip the Clark sensor in the measurement cell and wait for a steady signal
Add the nutrient and wait for a steady signal (respirometric curve)
Add the toxic and wait for a steady signal
Calculate $I_{tox} = \Delta I / \Delta n \cdot 100$
Procedure 2
Complete the procedure 1
Add formaldehyde and wait for a steady signal
Calculate $I_{in} = \Delta i / \Delta f \cdot 100$
Procedure 3
Disperse the yeast cells in the sample
Record a respirometric curve after an optimised incubation time
Record a respirometric curve using a blank sample
Calculate $I_{in} = (1 - \Delta s / \Delta b) \cdot 100$

Figure 2. Various respirometric curves recorded in both normal conditions and after exposure of the system to the toxic substance.

From an examination of the various respirometric curves recorded in both normal conditions and after exposure of the system to the toxic substance at several concentrations, we obtained a calibration curve to which reference can then by made to determine the toxicity of the selected sample. In practical terms, for each measurement two different respirometric curves are recorded: one in the presence of substrate alone and the other in the presence of substrate but after initial incubation with the toxic substance (for 8 min.); the results can by processed using one of the two following experimental data:

a) difference current intensity (δ), which is proportional to the 'distance' between the two respirometric curves, at a fixed time (30 min.) after the addition of the glucose;

b) the difference in amplitude of the angles (α - α_o) referring to the slopes with which the two respirometric curves attain a stationary state, which is measured 25 min. after the addition of glucose.

By this way it is possible to monitor integral toxicity of water from STP, especially if destined to potable uses, of soils incidentally polluted and followed during bioremediation, of sediments, and of industrial media. Two very actual applications were performed on radon pollution and cellular phone field effect on biological system, both of great actuality and object of controversies due to contrasting conclusions reached by different authors, very often based on unclear and measurements of poor accuracy. Particularly for cellular phone field it was concluded that 900 and 1800 MHz, corresponding to cellular phones of first and second generation, can be considered for some dangers to be used for exposure times longer than 5 hours. In any case a total reversibility was observed after 48 hours since the last exposure.

In all the respirometric biosensors very positive results on increasing analytical characteristics can be obtained with some modification able to improve the experimental systems.

2.5 Toxicity Biosensor Based on Human Tissue

In recent years, under pressure from the increasing public awareness of problems caused by the circulation of toxic compounds in the environment, several products have been reconsidered, also from the point of view of possible danger to man and their polluting effect on the environment.

The production and widespread use of hydrocarbons has attracted the attention of researchers, who have classified them among the most toxic substances. In particular, the US Environmental Protection Agency has classified aromatic hydrocarbons like benzene, toluene, the xylenes, etc, as highly toxic substances (Sacarello, 1994). Benzene, for instance, has been identified as carcinogenic agent that can induce acute myeloid leukaemia and other types of lymphoma in man (Sax and Lewis, 1989). Furthermore, these compounds were found to be included among the most widespread polluting substances found not only in the atmosphere but also in groundwater and soil; these three types of pollution coexist and are closely correlated.

Even though in many countries no "concentration limits" have yet been set by law for benzene, but only "quality objectives", this compound has been added to the list to be monitored in air, especially in cities, because of its use in green fuel and in the work environment, also as a result of the accumulating evidence of its carcinogenic properties (Sax and Lewis, 1989).

The standard reference method used far hydrocarbon analysis is gas chromatography (Manahan, 1984). Mass spectrometry is suitable for identifying even low levels of hydrocarbons, although it is normally too expensive to use for routine analyses. Another available technique is IR analysis (Manahan, 1984); finally, chemiluminescence (Manahan, 1984) is also sometimes used for aromatic hydrocarbon analysis.

Researchers of Singapore University (Tan et al., 1994) recently published the results of their work to develop a biosensor for benzene determination that satisfied the requirements of speed, cheapness, handiness and transportability and could be used as an alternative to the above mentioned analytical methods, often more rigorous but certainly more complex, costly and difficult to be used for in situ analyses. The above researchers used whole cells of *Pseudomonas putida*, which were immobilized by filtering the culture medium through a cellulose acetate membrane (Tan et al., 1994).

Our research group developed an original method for immobilizing whole cells on an agarized culture medium which was successfully used to produce toxicity biosensors based on immobilized yeast cells. It was therefore decided to immobilize whole cells of *Pseudomonas putida*, using this new method with a view to developing a biosensor for benzene determination (Campanella et al., 1996). The result confirm the possibility of assembling biosensors for the determination of benzene and other aromatic hydrocarbons using suitably immobilized colonies of *Pseudomonas putida*; the immobilization method used has a strong influence on the biosensor's analytical performance, especially its linear range, selectivity and response time. However, these last two characteristics probably also depend on the procedure used, during the growth phase, to select the cells and boost the growth of the *Pseudomonas* colony so as to be capable of biodegrading the benzene. The capacity to metabolize benzene or other aromatic hydrocarbons apparently depends on the presence of specific protein subunits forming part of the benzene dioxygenase enzyme contained in the cells of *Pseudomonas putida* (Zamanian and Mason, 1987) used. The interaction between the biological system and the environment has been studied aiming at the evaluation of the effects caused by natural and anthropogenic organic substances on different ecosystems. These evaluations allow on one side to individuate natural markers to be used for the biomonitoring of the environment and on the other side to study biochemical reactions occurring in the living organisms (target organs).

Humans, according to relatively recent bibliography (Parke and Williams, 1953) can partially metabolise benzene to trans, trans-muconic acid, so able to act as biomarker of benzene. So it is here presented a new biosensor to determine benzene based on human kidney tissue as this organ has an enzymatic content (dioxygenase) able to degrade benzene by the same way as *Pseudomonas*. The work was developed in two steps: firstly, the metabolic capacity of the above said enzymes toward benzene was verified; secondly, the biosensor was assembled, tested and applied.

The control of the degradation of benzene due to the kidney tissue was performed by a complex procedure based on the radioactive benzene marked with ^{14}C. If healthy and unhealthy (for instance cancerous) tissues are tested, differences in the metabolic capacity are recorded. So the human kidney tissue based biosensor is used not only in the traditional way to determine the substrate but to study the efficiency of the benzene biodegradation and so the health state of the kidney and consequently the toxicity to it of benzene. Due to its carcinogenic characteristics, benzene is supposed to be accumulated in cancerous tissues especially kidney tissues, due to the lost enzymatic activity and so to the partial or total absence of metabolic process So we tried to evidence this hypothesis on preparing and testing a biosensor completely similar to that one described, but in this case based on cancerous kidney tissue as biological component looking for any behaviour difference. Really the biosensor resulted much less active with a calibration slope of order of $10^{-4.5}$. In this case so the biosensor was unable with its response to yield any information about benzene concentration but it was able to furnish us much more important information about the lost health state of the tested tissue. Really the result ensures a good efficiency of the method to verify the differences in the degrading capacity between healthy and cancerous tissues. If it could act also in the preventive phase when tumour begins to form it should be a very attractive tool for medical and oncological aims.

The proposed sensor can be considered a new generation biosensor based directly on human tissue so avoiding this the aleatory transfer to human of data recorded on a different organism and at the same time to "measure" the state of a human organism.

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ADVANCED ENVIRONMENTAL BIOCHEMICAL SENSOR FOR WATER MONITORING

Automated Water Analyser Computer Supported System (AWACSS)

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Abstract: The protection of water resources and the secure delivery of clean water to consumers are important tasks for humans in the future. Therefore, it is necessary to have novel analytical systems to control water quality. Here we give a review on the performance of the AWACSS (Automated Water Analyser Computer Supported System) biosensor and describe the results obtained during a field test and for a validation with drinking water. The system was developed during a research project (EVK1-CT-2000-00045) supported by the European Commission under the Fifth Framework Programme and contributes to the implementation of the Key Action "Sustainable Management and Quality of Water" within the Energy, Environment and Sustainable Development. The system is based on immunochemical technology and can measure a variety of small organic pollutants in water at the low nanogram per liter level in a single few-minutes analysis without the need of any pre-concentration or pre-treatment steps. Furthermore, this instrument is equipped with a software package for remote control and web-based networking between the measurement and control stations, global management, trend analysis, and early-warning applications. The AWACSS biosensor is a fully automated, sensitive, cost-effective, and easy-to-use analytical system for the continuous monitoring of surface, ground and drinking water.

Key words: Automated Water Analyser Computer Supported System (AWACSS); fully automated biosensors; evanescent field; environmental analysis; trend analysis; monitoring

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1. INTRODUCTION

Pollution of water sources, aquifers and wetland systems caused by industry, agriculture, and municipally treated wastewater is a worldwide problem. Rivers, channels, lakes, oceans, and ground water are often contaminated by a variety of organic substances that can have adverse effects on aquatic life and pose risks to human health. As a result, this pollution of water resources has become a major task during the production of clean drinking water. Examples include herbicides, insecticides, fungicides, antibiotics, drugs, detergents, oils, industrial waste or by-products, endocrine disrupting compounds, and carcinogens. Within the European Union, new water-related environmental legislation clearly defines the need for regular monitoring of organic substances down to low nanogram per liter levels (2000/60/EC, 2000; 98/83/EC, 1998). Since many of the so-called priority substances to be monitored in water are rather difficult to analyze, European Union expert groups working on the implementation of the Water Framework Directive WFD (2000/60/EC, 2000) put considerable effort to propose a range of techniques and methods for their monitoring. True enforcement demands more frequent monitoring of water catchment areas and industrial plants need greater control of their wastewater to meet the demands of increased regulation.

The most prevalent technologies in water analysis are liquid chromatography (HPLC) and gas chromatography (GC) in combination with different detection principles. These well-established methods are looking back on decades of development and successful application but both require time-consuming enrichment of water samples by several orders of magnitude prior to analysis. This step in sample pre-treatment makes it difficult and rather expensive to develop suitable online monitoring systems and might be the reason why only a few systems exist so far (Proll et al., 2005).

Despite the fast developments of these techniques over the past decades, water-sector managers and researchers still strive for a water-monitoring device, which would be robust, cost-effective, automated and able to measure several tens of organic pollutants in parallel.

Another sought for feature is a possibility of instrument's remote control, automated data processing and generation of alarm signals when pollutant's concentration exceeds the pre-set threshold value. The use of such on-line sensors follows also new developments in the drinking water legislation and introduction of a risk assessment/risk management approach (World Health Organization WHO, 2003).

Immunochemically based techniques are a popular alternative to standard methods. Key features of immunoassays, such as their specificity, sensitivity and speed of analysis led to the development of numerous environmental applications (Barceló, 2000) and qualify them as suitable candidates to be used for environmental monitoring. Still, most of the commercially available techniques suffer from not fully described cross-reactivity of target analytes, matrix effects, limited availability of antibodies and not having capability of multi-analyte analysis (Oubina et al., 2000). Recently, the field of array-based technology for multianalyte detection has exploded. These techniques take advantage of the two-dimensional layout of recognition elements to allow simultaneous detection and quantification of multiple analytes (Rowe Taitt et al., 2002).

In the EU project AWACSS - Automated Water Analyser Computer Supported System (EVK1-CT-2000-00045) a multi-analyte immunoassay-based system has been constructed and successfully tested for analysis of aqueous samples (Tschmelak et al., 2005b, 2005c). The first article gives the reader an overview of the aims and scope of the AWACSS project as well as details about basic technology, immunoassays, software, and networking developed and utilized within the research project. The second article reports on the systems' performance, first real sample measurements, and an international collaborative trial (inter-laboratory tests) to compare the biosensor with conventional analytical methods.

Furthermore, several new ultra-sensitive immunoassays were developed for a variety of environmental pollutants and other substances of interest like estrone, progesterone, testosterone, bisphenol A, atrazine, propanil, caffeine, isoproturon, tetrahydrocannabinol, and several sulphonamides (Tschmelak, 2005). In case of estrone, a LOD below 0.2 ng/L and a LOQ below 1.4 ng/L was reached by reducing the amount of antibody per sample (Tschmelak et al., 2004b, 2005a). In case of propanil, an LOD of 0.6 ng/L below the one nanogram per liter barrier was reached for a single pesticide with a fully automated immunoassay (Tschmelak et al., 2004c). Additionally, by using this biosensor principle, it was possible to implement immunoassays for seven sulphonamides a LOD below 10 ng L^{-1} for each of the "antibiotics" (Tschmelak et al., 2004a).

2. EXPERIMENTAL SECTION

MATERIALS: Common chemicals of analytical grade were purchased from Sigma-Aldrich, Deisenhofen, Germany, or Merck KGaA, Darmstadt, Germany. The pesticides atrazine, isoproturon, and propanil were purchased as PESTANAL®, the antibiotic sulphamethizole and the hormone estrone were purchased as VETRANAL® analytical standards from Riedl-de Haën Laborchemikalien GmbH & Co. KG, Seelze, Germany. The polymer plasticiser bisphenol A was ordered from Sigma-Aldrich, Deisenhofen, Germany. The fluorescent dye CyDye™ Cy5.5 was purchased from Amersham Biosciences Europe GmbH, Freiburg, Germany. The aminodextrans Amdex™ with 40K and 170K Dalton molecular weight were purchased from Helix Research Company, Springfield, OR, USA. The dicarboxy-polyethylenglycole with 2K Dalton molecular weight was purchased from RAPP Polymere, Reutlingen, Germany. Labeling and purification of antibody were carried out as described in the product information sheet supplied with the labeling kit from Amersham Biosciences Europe GmbH, Freiburg, Germany. UV-Vis spectra were recorded using a Specord M500 spectrophotometer from Carl Zeiss Jena GmbH, Jena, Germany. The spatially resolved surface modification was performed using a parallel micro-dispensing system (TopSpot) with high-performance piezostack actuation system and integrated temperature adjustment from HSG-IMIT / IMTEK, Germany.

INSTRUMENTATION: The AWACSS instrument employs fluorescence-based detection of the binding of fluorophore-tagged biomolecules to the surface of an integrated optical waveguide chip (IO-transducer). The fiber-pigtailed chip, driven by a semiconductor laser, consists of a waveguide circuit, which distributes excitation light to 32 separate sensing patches on the chip surface. Bio/immuno-chemistry is used to sensitize each of the 32 patches to a specific analyte and a micro fluidic system is used to automatically handle the sample injection over the sensor surface, enabling rapid, simultaneous and high-sensitivity fluorescence detection of up to 32 pollutants. A fiber-coupled photodiode detection array is used to monitor the 32 separate fluorescence signals, and software has been developed for control of the optics and fluidics and data acquisition and processing for the fluorescence signals, laser power, and ambient and chip temperature. Key features of the instrument, chip fabrication, chip characterization, hardware requirements, and further details of the instrumentation are already described in the literature (Tschmelak et al., 2005b, 2005c). An HTC PAL auto sampler with modified cycle composer software (CTC Analytics AG, Zwingen, Switzerland) was used for dilutions, sample preparation (transferring 100 µL of the antibody stock solution to 900 µL of the sample followed by one or two mixing strokes) and the sample transfer to the AWACSS instrument. Liquid handling and data acquisition are fully automated and computer controlled. One measurement cycle with washing steps, injection of the sample and regeneration of the surface takes about 15 to 18 minutes.

IMMUNOCHEMISTRY: The immunochemistry utilized in this research takes advantage of a binding inhibition assay in heterogeneous phase that requires antibodies directed against specific analytes and analyte derivatives that can be covalently bound to a sensor surface (IO-transducer). The previously immobilized polymer-layer is used to reduce non-specific binding to the surface. A huge number of polyclonal antibodies and their corresponding analyte derivatives have been produced for a variety of organic micro-pollutants. After being purified and labeled with a dye (CyDye™ Cy5.5), they were developed into immunoassays and used in the project. The sample containing the analytes is incubated in a solution with the labeled specific antibodies. A 100-µL portion of the antibody stock solution is mixed with 900 µL of the sample by an auto sampler and the sample is then incubated for approximately five minutes. The antibodies bind to the analytes in a parallel competitive reaction, according to their affinity constants, during the incubation step until a well-defined condition of the reaction is reached. When the sample is pumped over the sensor surface, only the antibodies with free binding sites can bind to the surface. For the binding inhibition assay to be quantitative, the binding of the antibody to the surface must be mass transport-limited. This allows the signal to be a function of the diffusion rate to the surface and not of the kinetics of the surface binding. The number of high affinity binding sites on the surface has to be much higher than the number of antibodies used for one measurement. To be sure, that the binding is mass transport-limited, we use small amounts of antibodies and on the sensor surface we immobilize a huge excess of antigen derivatives.

DETECTION: The AWACSS instrument is based on evanescent field technology. Laser light is coupled into an optical transducer and guided down the Integrated Optical (IO)-chip. The transducer surface is chemically modified in spatially distinct loci with analyte derivatives. Analyte-specific antibodies are labeled with a fluorescent marker (CyDye Cy5.5™), which upon binding to the transducer surface are excited in the evanescent field. The emitted light is then collected for detection with 32 polymer fibers. The design allows for the simultaneous measurement of multi-analyte spots. Analyte recognition is based on a binding inhibition assay. Analyte derivatives are immobilized onto the transducer surface prior to the assay. Next, analyte-specific antibodies labeled with fluorescent markers are incubated with the analyte samples. After the short incubation period, the analyte solution flows over the transducer. Only analyte-specific antibodies with free binding sites will bind to the transducer surface whereas, at the same time, antibodies that have two analyte molecules bound to each epitope will not bind to the surface. The surface bound labeled antibodies are excited in the evanescent field and the fluorescence is detected. As a result, an inverse analyte signal is measured, with samples having low analyte concentrations giving rise to high fluorescence signals and samples with high analyte concentrations resulting in low fluorescence.

IMMOBILIZATION: Active esters were prepared with the derivatives, which are analyte molecules modified with a spacer containing a carboxyl group. Approximately 5.0 mg of the derivative were dissolved in 100 µL of dry *N*,*N*-dimethylformamide (DMF). *N*-hydroxysuccinimide (NHS) and *N*,*N*'-dicyclohexylcarbodiimide (DCC), each in 1.1-fold molar excess (referring to the amount of analyte derivative) were added to the solution. After stirring for several minutes, the solution was kept over night at room temperature. Finally, the solution was centrifuged (12,000 rpm) at approximately four degrees centigrade and the supernatant was stored under refrigeration. 50 mg aminodextran were dissolved in a mixture of 500 µL Milli-Q water and 500 µL DMF. The active ester solution was added, mixed thoroughly and kept overnight at room temperature. A ten-fold volume excess of methanol precipitated the aminodextran conjugate. The supernatant was removed and the conjugate was freeze-dried. The IO-chips were cleaned in a freshly prepared mixture (ratio 2:3) of hydrogen peroxide (30% H_2O_2) and concentrated sulphuric acid (65 % H_2SO_4) for approximately ten minutes and rinsed with Milli-Q water. After drying under a nitrogen flow, 25 µL of (3-glycidyloxypropyl)trimethoxysilane (GOPTS) were applied to the surface and reacted for up to 60 minutes. The silanized surface was rinsed with dry acetone and dried under a flow of nitrogen. Afterwards, the surface was imediately treated with 40 µL of a PEG solution (4 µg dicarboxy-polyethylenglycole per µL dichlormethane) and after evaporation incubated at 70 °C over night. The surface was then thoroughly rinsed with water and dried at room temperature under a flow of nitrogen. The activation of the PEG surface was carried out by applying a solution of 1 molar NHS and 1.5 molar DIC in DMF for 1 h. After rinsing with DMF followed by water and drying the chips were ready for the spotting procedure. Subsequently, the aminodextran conjugates were dissolved in Milli-Q water at a concentration of 1.0 up to 2.0 mg/mL and were immobilized by a parallel spotting device TopSpot from HSG - IMIT, Villingen-Schwenningen, Germany (www.hsg-imit.de) and IMTEK, Freiburg, Germany (www.imtek.de).

SAMPLING: A sampling system for on-line monitoring has been developed which allows direct intake of river water and its continuous transfer to the auto sampler of the AWACSS system. During the field test period, this developed sampling system was used. A schematic representation of the set-up is shown in Figure 1. The only river water pre-treatment step is the filtration using a five-micron size exclusion filter within the continuous monitoring experiment. Water in the flow through vessel with 80 mL volume was exchanged every three minutes adjusted by the pumping rate and the split valve.

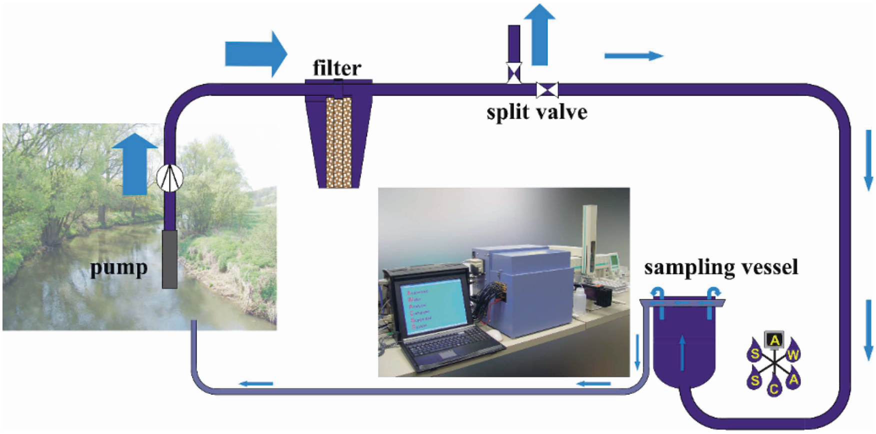

Figure 1. Schematic set-up of the sampling system for on-line monitoring which allows direct intake of river water and its continuous transfer to the auto sampler of the AWACSS system used at the first stand-alone field-test of the AWACSS instrument.

In a sampling experiment at River Rhine it could be shown that with this experimental set-up a life-time of three months could be achieved before an exchange of the filter cartridge becomes necessary because a blocking of the filter occurs, assuming that the average turbidity of the river water is 15 FNU (for River Rhine most often it is lower) and that a flow-rate of one liter per hour through the sampling vessel is applied. With this flow-rate the water in a 250 mL vessel is completely exchanged every 15 minutes.

MEASUREMENTS: For the measurements, we used polyclonal IgG antibodies from sheep and corresponding suitable analyte derivatives (haptens or similar substances). The entire sample volume was 1000 µL. For a calibration routine, 900 µL of spiked Milli-Q water was automatically mixed by the auto sampler with 100 µL of an antibody stock solution containing the antibodies and ovalbumin from chicken eggs (OVA) in ten-fold phosphate buffered saline (PBS) (ten-fold PBS: pH 6.8, 1500 mM sodium chloride, 100 mM potassium phosphate monobasic). After a defined incubation time of approximately five minutes this mixture was measured using the biosensor setup. The experimental design for a calibration routine consists of 12 independent blank (Milli-Q water) measurements and nine concentration steps (each measured as three replica) of the analyte (spiked

Milli-Q water). For all concentration steps and the blank measurements the mean value and the standard deviation (SD) for the replica was automatically calculated by the measurement control unit. The measured signal for the mean value of the blanks was set to 100% and all spiked samples could be obtained as a relative signal below this blank value. To fit the data set a logistic fit function (Dudley et al., 1985) (parameters of a logistic function: A_1, A_2, x_0, and p) with three free parameters (A_2, x_0, and p) was used. A_1, as the upper asymptote was fixed to 100% (relative signal for mean value of the blanks) and A_2 is the lower asymptote. The range between A_1 and A_2 is the dynamic signal range. The inflection point is given by the variable x_0 and represents the analyte concentration, which corresponds to a decrease of 50 % of the dynamic signal range (IC_{50}). The slope of the tangent in this point is given by the parameter p. In compliance with the IUPAC rules (The Orange Book) (Inczedy et al., 1998), the LOD is calculated as three times the SD of the blank measurements (SD_{blanks}) and the LOQ is calculated as ten times the SD of the blank measurements (SD_{blanks}). Statistical procedures and further calculations are included within the AWACSS evaluation software package. All real samples have been measured using the same system parameters as described for the calibration procedure.

3. RESULTS AND DISCUSSION

To validate the analytical performance of the AWACSS system under realistic field conditions, we calibrated the biosensor with a mixture of four analytes according to the multi-analyte protocol. The AWACSS IO-chip was modified with derivatives of atrazine, bisphenol A, estrone, and isoproturon according to the previously described immobilization strategy. Then a simultaneous calibration from 0 to 90 µg/L analyte concentration in Milli-Q water with mixed analytes and an antibody stock solution containing the four corresponding antibodies (anti-atrazine, anti-bisphenol A, anti-estrone, and anti-isoproturon) was performed. The amount of antibody was between 50 and 210 ng per sample. For all compounds, the calculated LOD is below 20 ng/L and all validation parameters, calculated relative signals, and standard deviations are summarized in Table 1 and Table 2. The resulting calibration curves are shown in Figure 2. This simultaneous multi-analyte calibration demonstrated the possibility to quantify pesticides from two different classes (triazines and phenylurea herbicides), endocrine disrupting compounds (bisphenol A), and steroid hormones (estrone), within one single measurement cycle, which only takes approx. 18 minutes. No cross-reactivity effect was observed for any of the tested analytes.

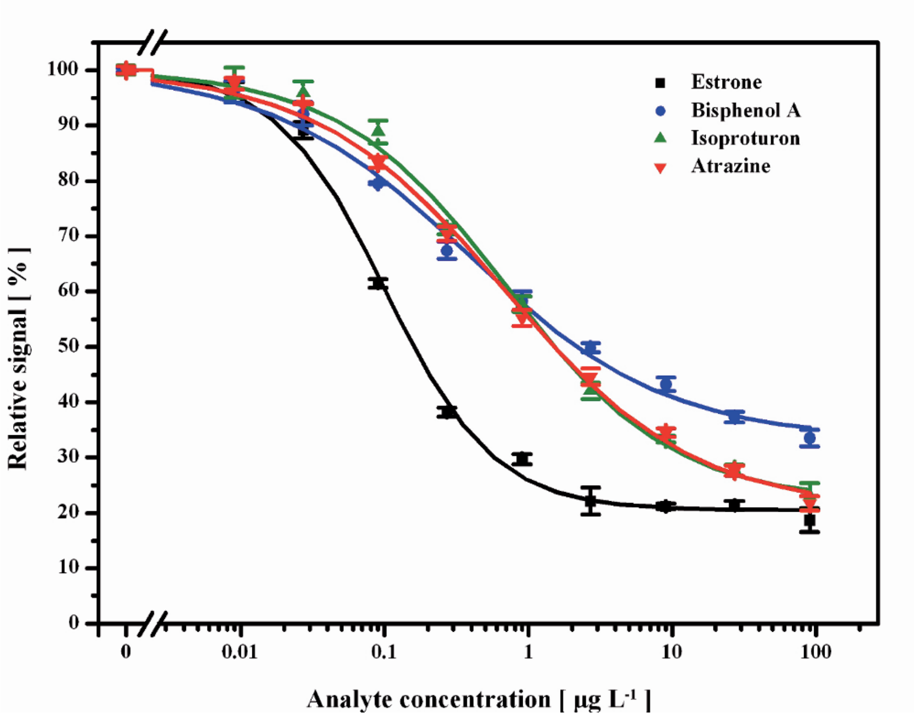

Figure 2. The resulting set of calibration curves for atrazine, bisphenol A, estrone, and isoproturon, which were measured in parallel on a multi-analyte transducer. For all compounds the calculated LOD is below 0.007 µg/L.

Table 1. Calculated relative signal values (%) with standard deviations for all concentration steps.

Concentration	Atrazine	Isoproturon	Bisphenol A	Estrone
0	100.00±0.66	100.00±0.83	100.00±0.33	100.00±0.38
0.009	97.56±1.07	97.74±2.69	96.14±1.98	96.01±1.87
0.027	94.00±0.16	95.92±2.04	92.14±2.13	89.17±1.47
0.09	83.31±1.01	88.87±2.12	79.58±0.20	61.53±0.82
0.27	70.44±1.28	71.19±0.86	67.45±1.50	38.25±0.87
0.9	55.24±1.45	57.75±1.39	58.25±1.85	29.74±0.90
2.7	44.65±1.53	42.13±1.46	49.88±0.75	22.15±2.45
9	34.51±0.78	33.41±0.55	43.29±1.17	21.22±0.47
27	27.66±0.89	27.77±0.99	37.37±0.95	21.37±0.80
90	21.76±1.28	23.01±2.42	33.61±1.55	18.74±2.12

Table 2. Resulting calibration parameters and validation parameters (LOD and LOQ) for all four analytes.

Parameter	Atrazine	Isoproturon	Bisphenol A	Estrone	Unit
A_1	100	100	100	100	[%]
A_2	20.76±2.04	22.08±5.67	33.37±2.16	20.55±2.49	[%]
x_0	0.68±0.09	0.70±0.28	0.38±0.07	0.10±0.03	[µg/L]
p	0.66±0.04	0.75±0.21	0.63±0.06	1.15±0.29	[ ]
LOD	0.003	0.007	0.001	0.003	[µg/L]
LOQ	0.018	0.040	0.004	0.007	[µg/L]

During the field testing the AWACSS biosensor was installed in a pipe bridge for drinking water production located at a riverside. River water was supplied to the sensor system via the sampling system, which has already been explained. During one day the fully automated system measured several times triplicates of river water samples and Milli-Q blanks as a reference. Based on the calibration parameters the individual concentrations of the four compounds were automatically calculated by the instruments software package. To validate the system all substances have also been analyzed by classical analytical techniques carried out by an accredited laboratory.

For estrone all biosensor measurements as well as the HPLC control measurements have been below the LOQ (LOQ-AWACSS 7 ng/L; LOQ-HPLC 1 ng/L) for both systems. Bisphenol A was not possible to measure because of an enormous leaching of this chemical substance out of the tubing of the sampling system. For the continuous monitoring of the two pesticides very good results could be obtained with the biosensor system which are approved by HPLC measurements as shown in Figure 3 for atrazine and in Figure 4 for isoproturon. Both pesticides appear in concentrations below the EU-limit for drinking water of 0.1 µg/L in the river water. In the case of the HPLC measurements for isoproturon the first data point was found to be below the LOQ of this method which indicates a non-significant measurement. Unfortunately, measurement number 5 of the AWACSS could not be used because of an air bubble covering the corresponding spot of the IO-chip during one measurement. Air bubbles coming with the river water into the sampling vessel caused this problem. In the future, this problem can be prevented by stopping the water flow through the sampling vessel before sample uptake.

This field test demonstrated the ability of the AWACSS to provide a tool for the continuous monitoring of organic pollutants in river water. Once a measurement station is installed only a little maintenance of the system is necessary. Non-experts can carry out the exchange of buffer solutions and the replacement of the sensor-chip unit after 500 measurements.

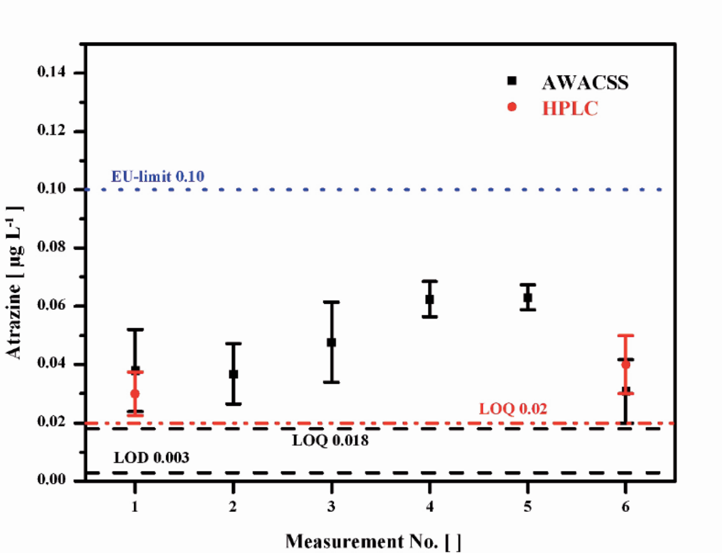

Figure 3. Results for biosensor-based atrazine monitoring (LOQ 0.018 µg/L) during the remote-controlled field test compared to HPLC (LOQ 0.02 µg/L) data.

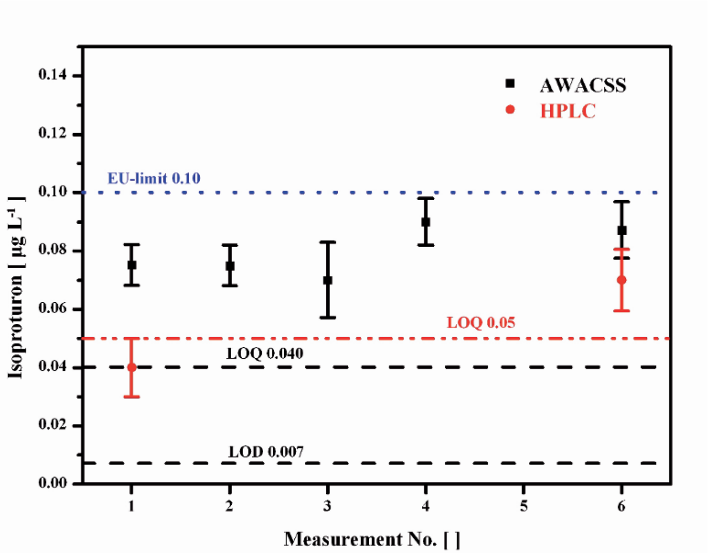

Figure 4. Results for biosensor-based isoproturon monitoring (LOQ 0.040 µg/L) during the remote-controlled field test compared to HPLC (LOQ 0.05 µg/L) data.

Having in mind actual needs of water-sector managers related to the implementation of the Drinking Water Directive DWD (98/83/EC, 1998) tap water should be used for the evaluation of the system performance. Therefore, the system was calibrated with a mixture of six analytes (atrazine, bisphenol A, estrone, isoproturon, sulphamethizole, and propanil) in parallel. The simultaneous calibration from 0 to 90 µg/L with mixed analytes and an antibody stock solution containing the six corresponding antibodies (anti-atrazine, anti-bisphenol A, anti-estrone, anti-isoproturon, anti-propanil, and anti-mixed-sulphonamides) was performed. The amount per antibody was between 50 and 210 ng per sample. The resulting calibration parameters and the calculated validation parameters are summarized in Table 3. The performance of the calibrated biosensor system was tested by analyzing spiked tap water at three different concentration levels. The spiking levels have been selected, especially for the pesticides, below, at and above the EU-limit of 0.1 µg/L for a single pesticide in drinking water. All concentration levels were measured in triplicate and referenced towards Milli-Q blanks. The found concentration values and the recovery rates are presented in Table 4.

Table 3. Multi-analyte assay performance (calibration parameters and LODs).

Parameter	A_1	A_2	x_0	P	LOD
Propanil	100	9.34±0.87	0.23±0.01	1.05±0.04	0.019
Atrazine	100	20.66±1.65	0.22±0.02	0.74±0.05	0.010
Isoproturon	100	8.39±0.52	0.17±0.01	1.23±0.04	0.020
Sulphamethizole	100	32.88±2.41	0.47±0.10	0.57±0.05	0.018
Bisphenol A	100	28.10±2.45	1.70±0.29	0.58±0.04	0.008
Estrone	100	16.72±1.05	0.09±0.01	1.46±0.12	0.007
Unit	[%]	[%]	[µg/L]	[ ]	[µg/L]

Table 4. Found concentration values including standard deviations and corresponding recovery rates (RR) for spiked drinking water at three spiking levels (SL).

	SL 0.5	RR	SL 1.0	RR	SL 1.5	RR
Propanil	0.048±0.008	96.7	0.080±0.004	80.0	0.174±0.008	115.8
Atrazine	0.043±0.009	86.7	0.087±0.017	87.0	0.167±0.015	111.5
Isoproturon	0.059±0.002	117.4	0.097±0.004	96.9	0.147±0.007	98.0
Sulphamethizole	0.055±0.003	110.8	0.086±0.032	85.6	0.164±0.015	109.6
Bisphenol A	0.016±0.002	31.6	0.144±0.013	143.8	0.153±0.037	101.8
Estrone	0.053±0.002	106.2	0.119±0.006	119.4	0.171±0.017	113.8
Unit	[µg/L]	[%]	[µg/L]	[%]	[µg/L]	[%]

As recommended by the AOAC International recovery rates (RR) should be in the range between 70 and 120 percent to match the requirements for a good analytical performance of a system. During this study the AWACSS succeeded this test with drinking water for propanil, atrazine, isoproturon, sulphamethizole and estrone. In the case of bisphenol A a contamination of the Milli-Q lab water with this compound could be identified as the reason of the insufficient RRs.

4. CONCLUSION

To validate immunoassays regarding robustness and precision usually samples representing different matrices have to be analyzed. The presented results from the AWACSS field test with river water together with the results obtained during the drinking water measurements clearly demonstrate that the analytical performance of this biosensor system is fully comparable to classical analytical techniques. All data have been treated according to IUPAC rules for pure and applied chemistry to obtain validation parameters for each analyte of the multi-analyte assays. The evaluation procedures for evaluating the analytical performance are in compliance to the recommendations given by the AOAC International. The results support the data of a round robin test mentioned within the introduction carried out during the AWACSS project where this system succeeded in measuring standardized samples including different matrices.

Because of the very low LODs in the lower ng per liter range - and even below - for many small organic compounds this system is not only useful as a monitoring device but also as a novel analytical instrument for trace analysis without the need of any sample pre-concentration. In addition, as a device for pre-screening of thousands of water samples analyzed by specialized laboratories per day, this system can help to reduce the costs per sample significantly. The employed immunochemistry based technique in principle can be used to analyze other aqueous samples coming from food safety, diagnostics or from the detection of other hazardous agents in the environment.

The AWACSS biosensor is a fully automated analytical system capable of multi-analyte detection and is ready to be used for the monitoring of different water bodies. Therefore, this system can be a very helpful tool for the implementation of the surface-water-monitoring programs that have to be defined by the end of 2007 by each EU member state.

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GENETICALLY ENGINEERED MICROORGANISMS FOR POLLUTION MONITORING

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Abstract: At the heart of every biosensor is a biological entity, the purpose of which is to react with the target analyte(s) and generate a readily quantifiable signal. Traditional biosensors are based on the unique specificity of enzymes to their substrates, antibodies to antigens or that of nucleic acids to their complementary sequences. In recent years we have promoted the use of a different concept, that of whole cell biosensors: natural or genetically engineered live cells that respond to pre-determined classes of chemicals. While some of the specificity characterizing molecule-based biosensors may be lost, it is more than compensated for by the fact that by using live cells we are able to detect, by very simple means, very complex series of reactions that can exist only in an intact, functioning cell. Only a sensor of this type can report on the "well being" of a system, on the toxicity of a sample, the genotoxicity of a chemical or the bioavailability of a pollutant. No molecular recognition or chemical analysis can provide this type of information. In order to turn such sensor cells into "real" biosensors, they need to be immobilized onto a solid platform and coupled into a signal transduction apparatus. Several avenues taken to accomplish this goal are described, including encapsulation in sol-gel, immobilization at the tips of optic fibers and integration into silicon chips.

Key words: biosensors; bioluminescence; fluorescence; genotoxicity; pollution monitoring; toxicity; whole-cell biosensors

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1. INTRODUCTION

There are two general approaches to the monitoring of chemicals in the environment. The traditional one is based on chemical or physical analysis: it allows highly accurate and sensitive determination of the exact composition of any sample, it is essential for regulatory purposes, and is necessary for understanding both the causes of pollution and the means for its potential remediation. However, a complete array of analytical instrumentation necessary for such exhaustive analysis is complex and costly, and requires specialized laboratories. In addition, such methodologies fail to provide data as to the bioavailability of pollutants, their effects on living systems, and their synergistic/antagonistic behavior in mixtures.

As a partial response to these needs, a complementary approach is based on the use of living systems in a variety of environmentally oriented bioassays. Numerous biological systems have been used for such purposes, ranging from live organism assays such as fish or *Daphnia* toxicity tests to others based on sub-cellular components or enzymes. All of them share the same characteristic: rather than **identify** the target chemical they assay its **effect**. A special position among the test organisms utilized for such purposes is held by unicellular microorganisms, bacteria in particular. Their large population sizes, rapid growth rates, low costs and facile maintenance often make them a lucrative option for pollution monitoring. An additional attractive characteristic is that bacteria can be genetically modified to respond by a detectable signal to pre-specified changes in their environmental conditions. In this article we describe this novel class of environmental monitoring tools: genetically engineered microorganisms "tailored" to respond in a dose-dependent manner to changes in environmental conditions.

Several recent reviews (Daunert et al., 2000; Kohler et al., 2000; Hansen and Sørensen, 2001; Belkin, 2003; Gu et al., 2004; Vollmer and Van Dyk, 2004) have addressed different aspects of the use of such genetically engineered microorganisms as environmental bioreporters. The present document highlights the most recent advances in this rapidly developing field.

2. BIOSENSORS, WHOLE-CELL BIOSENSORS AND ENVIRONMENTAL MONITORING

A variety of definitions can be found for the term "biosensor". A reasonably general one is "A device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" (IUPAC Compendium of Chemical Terminology, 1997). Normally, the obvious advantage in the use of biological material is in the extremely high specificity of biological molecules. Thus, successful biosensors have been based on the specific interactions between enzymes and their substrates, the recognition between antibodies and antigens, accessibility of specific target molecules to their receptors, or the high affinity of nucleic acids strands to their complementary sequences. In all of these, the focus is on the specificity endowed by the unique bio-recognition of two molecules.

The completely different analytical approach employed in our case calls for the biological entity in question to be not a molecule but rather a live, intact cell. Indeed, a lot of the specificity described above may be lost, but it is more than compensated for by the fact that by monitoring such an activity we are able to detect very complex series of reactions that can exist only in an intact, functioning cell. Global parameters such as bioavailability, toxicity, or genotoxicity cannot be probed with molecular recognition or chemical analysis; they can only be assayed by using live systems.

3. "LIGHTS OFF" AND "LIGHTS ON" ASSAYS

As implied above, the obvious strength of whole-cell biosensing is in not in the specificity of the observed responses but rather in their generality. This is most apparent in toxicity bioassays, designed to sum the negative impacts of the sample on a living system. In such assays, the question asked is not "what toxicants does the sample contain?" but rather "how toxic is the sample?"

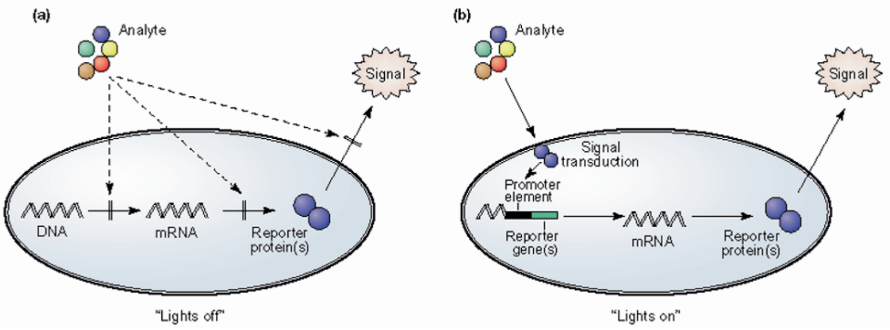

Figure 1. "Lights off" (A) and "lights on" (B) bacterial reporter assay principles. From Belkin (2003), with permission.

In recent years, genetically engineered microorganisms have played roles in two parallel research directions in the development of toxicity bioassays, which may be addressed as “lights off” and “lights on” assays. The differences between the two concepts (Belkin, 2003) are schematically represented in Figure 1.

The "lights off" concept is an extension of the widely accepted microbial toxicity bioassay, based upon measurement of the decrease in light emission as a function of sample concentration by the wild-type luminescent bacterium *Vibrio fischeri* following a short-term exposure to the sample (Quershi et al., 1998). A drawback often mentioned with regard to this assay is the marine origin of the test organism. Consequently, in the search for more “environmentally relevant” systems, other microorganisms were modified to constitutively luminesce and thus serve as possibly more realistic indicators of environmental toxicity. In this list one can mention *E. coli* HB101 (harboring *luxCDABE* of *V. fischeri*) immobilized in polyvinyl alcohol (Horsburgh et al., 2002), a *Pseudomonas fluorescens* transformed with the same plasmid (Tsiensing et al., 2002), or the cyanobacterium *Synechocystis* PCC6803 (marked with *luc* from the firefly *Photinus pyralis*, Shao et al., 2002). In the latter case the luciferase substrate – firefly luciferin – had to be added externally. Using a different approach, Ulitzur et al. (2002) report the use of a highly sensitive variant of the marine bioluminescent *Photobacterium leiognathi*; Weitz et al. (2002) demonstrate the use of two naturally bioluminescent fungi, *Armillaria mellea* and *Nycena citricolor*, for the same purpose.

A different approach to pollution-effect bioassays is based upon the molecular fusion of a reporter system to selected gene promoters of different stress response regulons. Assuming that no single reporter strain will be able to cover all potential cellular stress factors, it has been proposed that a panel of such strains be used (Belkin et al., 1997). Similar panels have recently been shown to sensitively respond to important environmental pollutants such as dioxins (Min et al., 2003) and endocrine disruptors (Gu et al., 2002). In these and many similar studies, the reporter system of choice was a microbial bioluminescence operon (*luxCDABE*); hence the reference to the “lights on” assays (Figure 1). In all cases, the genetic stability of the strain or the reporter fusion was not reported to present a problem.

In a similar manner, bacterial strains have also been developed for assaying genotoxicity, rather than “regular” toxicity. In these cases, the promoters serving as the sensing elements were selected from among DNA repair operons such as the SOS system, and the reporters were either bacterial *lux* or *β-gal* (Kohler et al., 2000). Recent reports propose the use of a fluorescent protein gene – *Aquorea victoria* gfp – as an alternative reporter system (Kostrzynska et al., 2002; Sagi et al., 2003). A more developed yeast-based (*Saccharomyces cerevisiae*) system for genotoxicity assessment is being continuously improved upon by Walmsley and coworkers (Knight et al., 2004).

4. DETECTION OF SPECIFIC POLLUTANTS

Since the pioneering work of Sayler and coworkers in the construction of a *lux* fusion for the specific detection of naphthalene and salicylate (Burlage et al., 1990), there has been a steady stream of similar constructs responsive to distinct organic or inorganic pollutants or classes of pollutants (For in-depth reviews: Daunert et al., 2000; Kohler et al., 2000; Hansen and Sørensen, 2001; Belkin, 2003; Gu et al., 2004; Vollmer and Van Dyk, 2004). Bioluminescence has served as the reporter in most cases, with a few examples of β-galactosidase activity and – more recently – GFP accumulation. Recent additions to these bio-reporter families include a bioluminescent phenol-sensing *Acinetobacter* (Abd-El Haleem et al., 2002), a GFP-based toluene-responsive *Pseudomonas fluorescens* (Stiner and Halverson 2002) and a *β-gal* reporter of 3-chlorocatechol (Guan et al., 2002). Prominent among the analytes detected in this manner over the last decade are heavy metals, with numerous variations, from mercury detection by *E. coli* (Selifonova et al., 1993) to As, Fe, Pb and Cd sensing in *Saccharomyces cerevisiae* (Radhika et al., 2005). A different class of pollutants that naturally lends itself to detection by microbial reporters is antibiotics. Bahl et al. (2004) have recently demonstrated fluorescent detection of tetracycline in a rat intestine using an *E. coli* reporter.

It should be borne in mind that almost with no exception microbial reporters are likely to provide less sensitive and accurate detection and quantification of specific pollutants than standard analytical chemical techniques (Kohler et al., 2000). Possibly the main advantage offered by whole-cell sensors to specific pollutants is that they detect only the bioavailable fraction of the target chemical, thus allowing its differentiation from the non-available one. Such information may be highly valuable for risk assessment, as well as for the comparison and design of remediation options.

5. NUTRIENTS BIOAVAILABILITY

An interesting offshoot of environmental promoter-reporter fusions was the construction of bioreporters of nutrient bioavailability. Although wastewater nitrogen and phosphorus are not the first on the “most wanted” pollutant lists, they are nevertheless a primary cause of eutrophication in aquatic environments and the direct trigger for the development of algal and cyanobacterial blooms. It thus seemed to be of interest to see how much of the limiting nutrients is actually "seen" by phytoplankton cells, as opposed to the total concentration determined by chemical means. Using similar strategies, Gillor et al. (2003) and Mbeunkui et al. (2002) have reported on a *Synechococcus* sp. with a *glnA::lux* fusion and a *Synechocystis* sp. with a *nblA::lux* fusion, respectively, both of them sensitive reporters of bioavailable nitrogen. The former was responsive to ammonia, nitrate, nitrite and organic nitrogen, whereas the latter was characterized mostly for nitrate detection. Similarly, Ivanikova et al. (2005) have reported a *Synechocystis* strain with the NtcA/B-dependent.

Nitrate/nitrite-activated *nirA* promoter fused to bacterial *luxAB* that generates nitrate-dependent bioluminescence. Gillor et al. (2002) have also reported a highly sensitive phosphorus bioavailability sensor based upon a *phoA::lux* fusion in *Synechococcus* sp. Using the latter strain it was demonstrated that under phosphorus limitation, "bioavailable P" constituted 1% or less of the chemically determined element in a freshwater lake (Gillor et al., unpublished). Dollard and Billard (2003) and Taylor et al. (2004) have demonstrated similar P- and N-responsive constructs in *E. coli,* the former using a *phoA* promoter fused to *lux*, the latter a *nar* promoter fused to GFP. An additional element highly significant to primary producers is iron; to assess the bioavailability of Fe in a freshwater environment, Durham et al. (2002) have fused *Vibrio harveyi luxAB* genes to the *isiAB* promoter of *Synechococcus* PCC 7942 and reported a sensitive dose-dependent response.

6. SELECTION OF REPORTER FUNCTION

Among the genes used over the years as the reporting elements in environmental microbial sensor systems, bacterial bioluminescence played a most prominent role. Several bioluminescence reporter systems are available, some of them recently compared by Mitchell et al. (2005). During the last few years, as increasingly versatile fluorescent protein genes became available for general use (Southward and Surette, 2002; Zhang et al., 2002), their popularity as reporters increased as well. At least two studies (Hakkila et al., 2002; Sagi et al., 2003) compared bioluminescent to fluorescent reporting and came up with similar conclusions: bioluminescence allows a much faster and more sensitive detection of the target analyte than fluorescence. The advantages of bioluminescence over fluorescent reporting are not surprising, as the former is a measure of enzymatic activity whereas the latter quantifies the presence of the protein. However, a unique advantage offered by GFP and other fluorescent proteins is their stability. When real time monitoring of the developing signal is impractical, fluorescence may prove to be much more reliable; if sufficient time is allowed for signal accumulation, even the sensitivity will be significantly improved (Sagi et al., 2003). Recent improvements in available fluorescent protein reporters and in detection technologies appear to be closing this gap. Indeed, Wells et al. (2005) have demonstrated arsenic-inducible eGFP production in single *E. coli* cells. Another attractive enzymatic reporting option is based on electrochemical rather than optical detection: with a correct use of substrate, the product of the activity of the reporter enzyme can be detected electrochemically. This has been utilized for rapid and sensitive detection of heavy metals and other toxicants (Biran et al., 2000; Paitan et al., 2004), and has recently been miniaturized and incorporated into a silicon chip (Popovtzer et al., 2005).

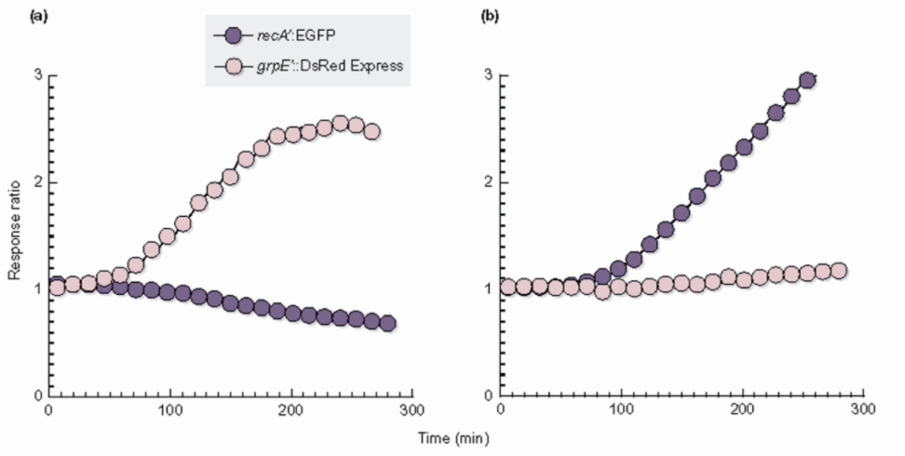

Figure 2. Responses to a genotoxicant (nalidixic acid, 6 mg/L) and to a "regular" toxicant (ethanol, 6 %) of a double-labeled *E. coli* reporter. The plasmid in this construct harbors both a *recA'::egfp* (green fluorescence; excitation 488 nm; emission 535 nm) and a *grpE'::dsred* Express (red fluorescence; excitation 558 nm; emission 583 nm). Fluorescent protein genes were obtained from BD Biosciences Clontech. From Belkin (2003), with permission.

7. DUAL LABELING

There have been very few reports on the inclusion of two distinct reporters in a single organism. In an early study, Wood and Gruber (1996) introduced two beetle luciferases, different in their light emission spectra, into *E. coli*. A similar study was recently reported in which two fluorescent proteins (GFPuv and YFP) were introduced into the same bacterial host species (Mirasoli et al., 2002). In both cases one of the reporters served as the responder to the analyte, and the other as an internal control. A different approach was used by Mitchell and Gu, who reported on a bacterial reporter strain that responds by fluorescence to DNA damage hazards and by luminescence to oxidative stress (Mitchell and Gu, 2004).

We have recently constructed a different double-labeled *E. coli* construct, containing two inducible promoters fused to different fluorescent protein genes: *recA'::egfp* and *grpE'::dsredExpress.* The *recA* promoter is a part of the bacterial SOS system and its activation is considered an indication of DNA damage hazard (Vollmer et al., 1997). GrpE is a heat shock protein, induced by a broad spectrum of chemicals, and was shown to be an excellent indicator of general toxic cellular stress (Van Dyk et al., 1994). Their combination, therefore, potentially allows an assay of both genotoxicity and "regular" toxicity by the same organism. Indeed, when separately challenged with model chemicals, nalidixic acid (an SOS activator) and ethanol (a heat shock inducer), the strain responded by an enhanced expression of the expected protein (Figure 2).

8. IMMOBILIZATION AND INTEGRATION INTO BIOSENSORS

Regardless of the sophistication of its genetic engineering, the applicability of a bacterial reporter strain may be limited to its use as a laboratory reagent. To be taken outside these boundaries, it needs to be incorporated into a biosensing device that will allow storage and maintenance of the live cells, sample introduction, and signal transduction. Several recent reports offer some potential incorporation solutions including alginate attachment onto optic fiber tips (Polyak et al., 2001), agar immobilization at the bottom of microtiter plate wells (Mbeunkui et al., 2002) and encapsulation in sol gel matrices (Rajan Premkumar et al., 2002). In the latter report, encapsulated luminescent bacteria maintained full activity at 4 ^{o}C for over 3 months. A different approach, that of random embedding of individual fluorescent *E. coli* and *Saccharomyces cerevisiae* cells into a high-density microwell array etched at the distal edge of an optical imaging fiber was reported by Biran and Walt (2002). The location and fluorescence of each individual cell was monitored using an optical decoding system, based upon the specific "signature" of each cell type. A chip-based system was developed by Simpson and coworkers, who used a CMOS (complementary metal-oxide semiconductor) imager for very l ow-level detection of the bioluminescent signal of a *Pseudomonas fluorescence* strain induced by naphthalene or salicylate (Bolton et al., 2002;

Sayler et al., 2004; Nivens et al., 2004). Their device, termed bioluminescent bioreporter integrated circuit (BIBIC), is probably be the first integrated whole-cell biochip.

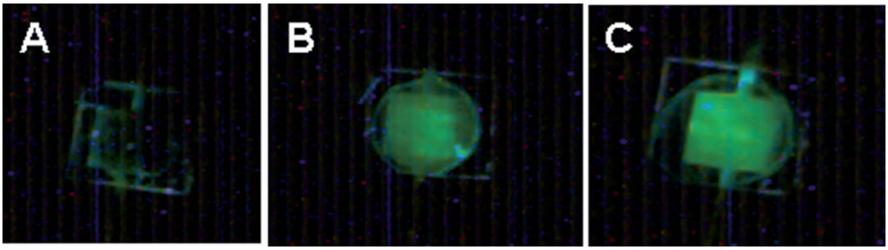

Figure 3. On-chip images of an E. coli strain containing a *recA'::egfp* fusion, non-induced (A) or induced by 1 (B) or 3 (C) mg/L nalidixic acid. Images were taken 3 hours after induction. Cavity area was 1 mm^2 and it contained ca. 10^7 cells.

A different tack towards the production of a whole-cell biochip is pursued in our laboratory, in which fluorescent *E. coli* sensor cells are maintained in mm-size cavities on a silicon chip. The fluorescence induced following an introduction of the sample into the cavity is recorded by a CMOS imager and quantified. Figure 3 displays an on-chip image of a *recA'::egfp* harboring strain, induced by nalidixic acid.

9. WHOLE-CELL ARRAYS

Microarrays have revolutionized our ability to characterize and quantify biologically relevant molecules. A large family of well-defined reactive molecules is fixed onto a mapped solid surface grid, and exposed to a multi-component analyte mixture. Sites in which a recognition event has occurred (such as by a complementary nucleic acid sequence) are identified by one of several possible detection techniques (e.g. fluorescence). The characteristics of the sample - and hence the constituents and/or the response of the studied system - can then be discerned from the nature of the bioreceptor molecules occupying these sites. Using this principle, an increasingly large number of applications are being developed in medicine, biology, toxicology, drug screening and more.

Most of the arrays described are based on oligonucleotides (Wang, 2000; Barrett and Kawasaki, 2003), some on antibodies, proteins (Lee, 2001; Barry and Soloviev, 2004; Lueking et al., 2005) or enzymes (Park and Clark, 2002). A few years ago, the idea of **whole-cell arrays** has been advanced by Van Dyk and coworkers (2001) who described the LuxArray: a collection of 689 non-redundant functional promoter fusions to *Photorhabdus* *luminescens luxCDABE* in live *E. coli* strains, representing close to 30% of the predicted transcriptional units in this bacterium. High-density printing of the reporter strains to membranes on agar plates was used for simultaneous assays of gene expression with impressive results. Initially aimed towards gene expression studies, there is no doubt that this approach can be successfully utilized for environmental monitoring, efficiently combining effect-testing with analyte identification. Descriptions of such arrays have recently been published by Lee et al. (2005), Biran et al. (2003) and by Kuang et al. (2004), the former utilizing miniature agar-immobilized culture drops and the latter two using individual cells attached to the end of an optic fiber bundle.

10. SUMMARY

The last decade has witnessed the development of a novel class of environmental monitoring tools: genetically engineered microorganisms that respond in a dose-dependent manner to the presence of toxic pollutants. Recent advances in the field include the expansion of available reporter functions (with multi-colored fluorescent proteins), a broadening of the detected chemical effects (such as the availability of nutrients) and enhancement of the spectrum of reporter microorganisms to include cyanobacteria, yeast and fungi. Most importantly, the stage has been set for the incorporation of such cells into various whole-cell array formats including silicon chips, optic fibers and other configurations.

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SOME ADVANCES IN ENVIRONMENTAL ANALYTICS AND MONITORING

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Abstract: The level of knowledge concerning the state of the environment increases continually. Selected examples of activities of the Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology (Gdańsk, Poland) are presented. Among them the presence of pharmaceutical residues in the aquatic environment has gained specific attention, which led to the development of on-line and off-line SPE enrichment procedures followed by LC-DAD-MS analysis. A new solvent filled passive sampler, which can cover a long sampling period integrating the pollutant concentration over time, has been presented. The method for simultaneous determination of selected phenols and phenoxyacids based on their preconcentration using solid-phase extraction and analysis by reverse phase HPLC has been presented and its application for the monitoring of pollutants in the coastal waters of the Gulf of Gdansk (Baltic Sea, Poland) is also shown. Data concerning degradation processes of selected polycyclic aromatic hydrocarbons in various organic solvents are shown. Degradation can not be eliminated entirely, but the process can be slowed down considerably. Some degradation products were identified using LC-DAD-MS with a single quadrupole. Application of a LC-ES-MS technique for the determination of anatoxin, nodularin and microcystins in Polish lakes and the sea has also been presented.

Key words: analytics; pharmaceutical residue; passive sampling; cyanobacteria; phenoxyacids; chlorophenols; HPLC-DAD-MS; degradation; water analysis; on-line SE; kinetic study

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1. INTRODUCTION

According to more and more common opinion, analytics and monitoring constitute the two pillars on which all of environmental science is based. It is true in the situation when both analytics and monitoring as such do not directly solve any of the problems concerning the environment. They are powerful tools only useful for gaining information about the state of the environment and phenomena and processes undergoing there (Namieśnik, 2000). With the purpose of making analytical information as reliable as possible, quality control and quality assurance play particularly important roles.

Measurement devices used for either analytical or monitoring purposes differ in a great manner. The basic differences between these are presented in Table 1.

Table 1. Short description of features that measurement devices used for environmental analytics and monitoring possess.

Parameter	Short description
Response time	-Analytical information "produced" in real time or with only a short time delay.
Resolution of results	- Very high
Automation level	- Very high - Long time of so-called autonomous work
Specific technical requirements	- Automatic calibration and zeroing of instruments - Provision of work of device against sudden interpretation of power supply - Equipment of the instrument with: independent power supply, separate calibration module, system of filling up of cells of electrochemical detectors, device protecting out of the flame (monitors based on FID and FPD detectors)

Suitable analytical information is a final result of work carried out both *in situ* and in the laboratory. Analytical chemists are able to provide to environmentalists different types of information among which we can emphasize concentration of an analyte and its time and space fluctuations, distribution of an analyte inside the material object under investigation, total content of an element and its speciation, confirmation of the presence of a given species in a sample as well as elucidation of the presence of an analyte at the concentration level above the threshold limit value TLV. The last two decades constitute a period of rapid development of environmental analytics and monitoring. Two main tendencies can be observed; first of all is the development and validation of new analytical procedures and the second one is the design, construction and application of new types of measuring devices.

The level of knowledge concerning the state of the environment increases constantly. Information about new pollutants found in environmental samples is published in many journals. The attention of analysts is oriented towards non-regulated pollutants, also called either new emerging pollutants, non-target pollutants or non-identified pollutants.

In the next part of this paper, some examples of the activities of the Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology (Gdańsk, Poland) are presented.

2. PHARMACEUTICAL RESIDUES IN AQUEOUS SAMPLES

The presence of non-steroidal anti-inflammatory drugs (NSAIDs) in the aquatic environment has raised great concern (Löffler and Ternes, 2003; Dębska et al., 2004). An automated method for the determination of NSAIDs in natural waters using on-line enrichment based on SPE with cartridges, which are in the form of a precolumn, has been described and compared with the same chromatographic method using off-line SPE. The performance of both procedures was tested with real water samples.

For off-line SPE of analytes from water samples different sorbents were applied, out of which the following gave satisfying results: Strata X (Phenomenex), Narc-2 (J.T.Baker), SDB-1 (J.T.Baker), C18 Polar PLUS (J.T.Baker), Quaternary amine (Merck), calixarene (home made sorbent), Lichrolut RP-18 (Merck), Discovery (Supelco), and DSC-18 (Supelco). Results were compared as recoveries (%) of NSAIDs calculated on the basis of LC-DAD-MS analysis of extracts of spiked tap water. For all selected NSAIDs C18 sorbent gave the highest recoveries (from 91 to 99%). Also, relative standard deviation values were low - in all cases lower than 8.0%. No breakthrough was observed.

In the on-line technique, enrichment of analytes from water samples was performed using 10 mm × 2 mm cartridges with a low-pressure six-port valve connected on-line to the chromatographic system. The automatic column-switching device used in the SPE process is illustrated schematically in Figure 1.

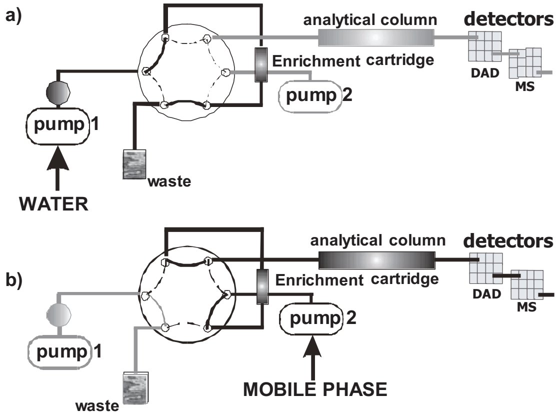

Figure 1. Schematic view of the described on-line SPE-HPLC-DAD-MS system: a) valve position during enrichment step; b) valve position during elution and analysis step.

In order to compare the method performance of on-line and off-line SPE procedures developed for selected pharmaceutical residue in water, Table 2 summarizes several indicative parameters for both methods.

Table 2. Comparison of basic analytical parameters characterizing on-line and off-line SPE procedure developed for selected pharmaceutical residues in water.

	Type of SPE enrichment technique			
	Off line		*On-line*	
	DAD detection	MS detection	DAD detection	MS detection
Repeatability (RSD %)	<11(3.3-10.4)	<21(9.6-20.4)	<7 (0.2-6.4)	<17(5.4-16.8)
Accuracy (% recovery)	32.3-100.4		89.2-105.1	
Spiking concentration	0.1-10 µg/L		0.01-1 µg/L	
Linearity (r^2)	0.999	0.995	0.999	0.996
Calibration range	0.1-150 µg/L		0.01-100 µg/L	
Sensitivity (LOD)	20-950 ng/L		0.7-94 ng/L	

In the on-line procedure, total time is about 1 hour, while in case of the off-line procedure, 8 hours should be spent to get a result. The performance of the method was tested on real samples using LiChrolut RP-18 as a sorbent. The recoveries for real samples were similar to the ones obtained when Milli-Q water was used and reached 80-95% (Dębska et al., 2005).

Many different water samples were analyzed in order to verify methods, which have been evaluated by us. Particularly, samples taken from lakes and rivers in North Poland and a seawater sample collected along the seashore of the Gulf of Gdańsk (South Baltic) were analysed. Commonly, in most of the water samples, which have been analyzed, diflunisal was presented at the concentration varied from 0.038 μg/L up to 0.123 μg/L. Generally lake waters were more polluted with NSAIDs than the others. Also river water was affected by analyzed compounds (minimal concentration founded was 0.055 μg/L for fenoprofen and maximum concentration was for diclofenac 0.390 μg/L). None of the water sample contained either tolmetin or naproxen. The presence of selected NSAIDs in some of the samples can possibly result from neighborhood of the district with many small houses.

3. PASSIVE SAMPLING METHOD FOR LONG-TERM MONITORING OF ORGANIC POLLUTANTS IN WATER BODIES

Passive dosimetry, until now widely used to monitor air pollutants, has been applied to monitor organic contaminants in water. Contrary to dynamic techniques, the passive type of sampling method is less sensitive to extreme variations of the organic pollutant concentration in natural waters. A passive sampler can cover a long sampling period integrating the pollutant concentration over time. Since only a few analyses are necessary over the monitoring period, analytical costs (usually connected with expensive dynamic sample isolation and preconcentration techniques) can be substantially reduced. Moreover, decomposition of the sample by transport and storage and/or changes during the sample enrichment step is also minimised (Śliwka-Kaszyńska et al., 2003).

Several designs of membrane samplers have been proposed (Namieśnik et al., 2005; Kot et al., 2000), including: solvent filled devices; semipermeable membrane devices (SPMDs); passive in situ concentration/extraction sampler (PISCES); supported liquid membrane (SLM) technique; and sorbent filled devices. We have developed another type of passive sampler, 2 cm in diameter, equipped with a membrane through which pollutants migrate to be absorbed by an organic solvent filling the device (Kot-Wasik, 2004). The scheme of sampler is shown in Figure 2.

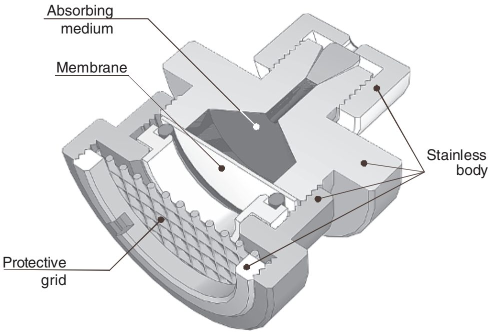

Figure 2. A cross-view of passive sampler.

After the passive sampler has been exposed for a certain period of time (varied from 30 to 60 days) the solvent is analysed by chromatography without any further pre-treatment. Samples are collected by inserting the syringe needle inside the chamber, followed by drawing an appropriate volume of extract into the syringe.

Concentrations of analytes inside passive samplers are shown in Figure 3.

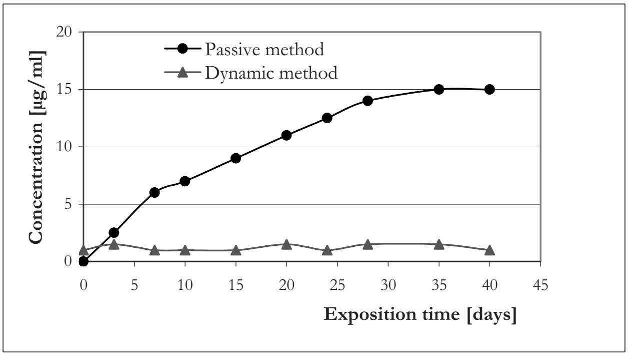

Figure 3. Changes of pentachlorophenol concentration inside (measurement based on passive sampling) and outside (measurement based on dynamic sampling using SPE technique) a passive sampler (Dąbrowska et al., 2004a).

On the basis of the results of our studies, it is possible to distinguish some specific groups of analytes, which have been described in literature (Dąbrowska et al., 2004a). Thus, all compounds that possess a functional group (with an exception to the electron donor hydroxyl group-*OH*) which pulls out electrons from the aromatic ring, e.g. Cl^- or NO_2, will migrate at the fastest rate. The second condition necessary for the enrichment of organic compounds in the passive sampler filled with cyclohexane or isooctane is that the logarithmic value of partition coefficient between octanol and water is larger than 3. Moreover, the larger the partition coefficient, the higher enrichment factor. Compounds with a low partition coefficient value but with very good solubility in water and having an electron donor group in the aromatic ring, do not migrate inside the passive samplers.

The passive sampler was used in the environmental monitoring of the pollution of Polish lakes (10 different lakes in Pomorskie voivod, North Poland) that are in most cases very interesting for tourists. During 2000 and 2001 passive samplers were exposed in two seasonal periods: spring and autumn time giving data concerning the presence of atrazine, simazine, pentachlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,6-trichlorophenol and dinoseb in the lake waters. No dinoseb was detected either in spring or in autumn time, while dichlorophenols were detected nearly in all samples collected in autumn time. Pentachlorophenol was found in lakes situated close to the Gdańsk area. The highest detected concentration was 48 μg/L for 2,6-dichlorophenol in autumn 2000. Atrazine and simazine were detected occasionally (2 times in the spring period) and their concentration was close to the detection limit. The leaching processes from surrounding agricultural areas may be responsible for the fact that pesticides were found in passive samplers exposed in lakes during springtime. Chlorophenols presented inside passive devices during autumn time might be present because they come from degradation of phenoxy acid herbicides applied in spring, however industrial activity can be responsible for the pollution of lakes, too.

4. DEGRADATION STUDIES

The degradation of organic compounds in the environment can take place with the help of microorganisms and enzymes, under aerobic and anaerobic conditions. In the majority of cases, a degradation of the basic structure of the compound takes place which will lead to a complete decomposition to carbon monoxide, water and inorganic salts (Dąbrowska et al., 2004a).

Three aspects should be taken into consideration if the influence of degradation processes on the reliability of analytical data should be considered: (1) the influence of degradation processes on the concentration of an analyte in the environment, (2) the influence of the same processes on the stability of an analyte in handled samples (at the step of transport and storage) and (3) the influence of degradation processes on the stability of concentration levels of an analyte in extracts and solutions obtained during the sample pre-treatment prior to the final analysis.

4.1 Photodegradation

Relatively little is known of the photochemical reactivity of polycyclic aromatic hydrocarbons (PAHs) when they are present in organic solvents. Generally, photodegradation of PAHs in solutions is an oxidative process. The more polar the solvent is, the faster is the degradation process of PAH. The rate of the degradation may also be affected by the amount of dissolved oxygen, temperature and light intensity.

The monitoring of the degradation of PAHs in selected organic solvents showed that the degradation of benzo(a)pyrene (BaP) (%) decreased in the following order: dichloromethane >acetonitrile >hexane ≥cyclohexane >methanol. This order may possibly be related to the solubility of oxygen in a solvent. The data obtained fit well with the first-order rate equation (Figure 4).

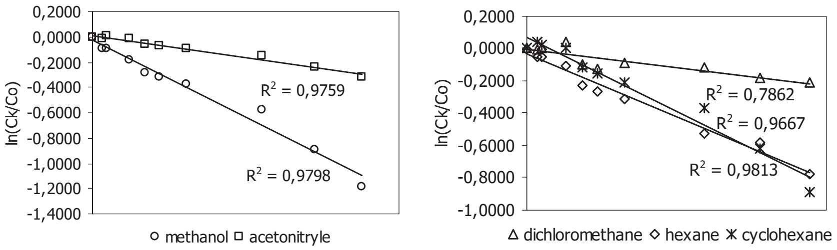

Figure 4. Graphical presentation of dependence of degradation process of benzo(a)pyrene in different solvents.

4.2 Biodegradation

The degradation of BaP is consistent with the ability of microorganisms to utilize the compound as a carbon source for growth. In natural waters, biodegradation of PAHs is enhanced by photodegradation, especially within the surface and sub-surface layers (up to 2 m for transparent waters). Biodegradation of BaP monitored in different types of natural waters, is shown in Figure 5.

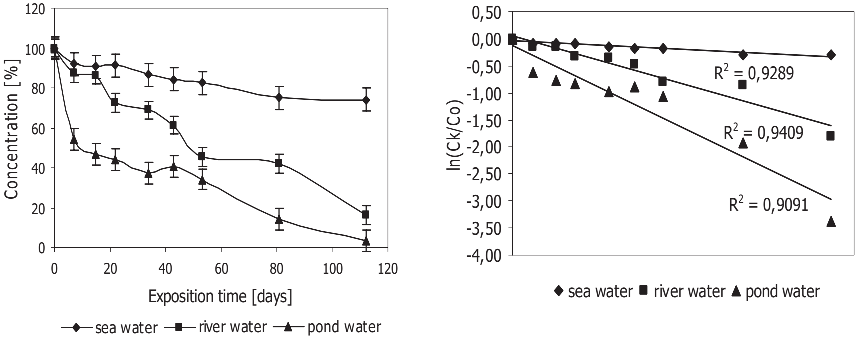

Figure 5. (A) Graphical presentation of the dependence of the process of degradation of benzo(a)pyrene in different natural water samples; (B) the first order reactions kinetics calculated for different natural water media.

The observed degradation rates of BaP in natural waters were in the following order: pond water > river water > seawater. In seawater samples from the Gulf of Gdansk the BaP degradation started later in comparison with pond water and the rate of degradation was significantly slower. Most likely it is so because of microbial flora from the sea environment which has been continuously exposed to oil derivatives (PAHs).

4.3 Degradation Products

The conversions taking place in solutions of fluorene in organic solvents result in the formation of 9-fluorenone and 9-hydroxyfluorene. Anthracene turned out to be the compound that very quickly underwent photodegradation, yielding anthraquinone as the main degradation product. Photodegradation of BaP performed under conditions as comparable to those naturally occurring (as possible) resulted in a number of BaP degradation products as presented in Figure 6.

In the case of compound 1, UV and MS spectra pattern suggest that BaP-4,5-dihydrodiol has been detected. Compound 2 has been identified as 2-hydroxy-BaP-1,6-dione ($C_{20}H_{10}O_3$; M=298 g/mol) (Dąbrowska et al., 2004b).

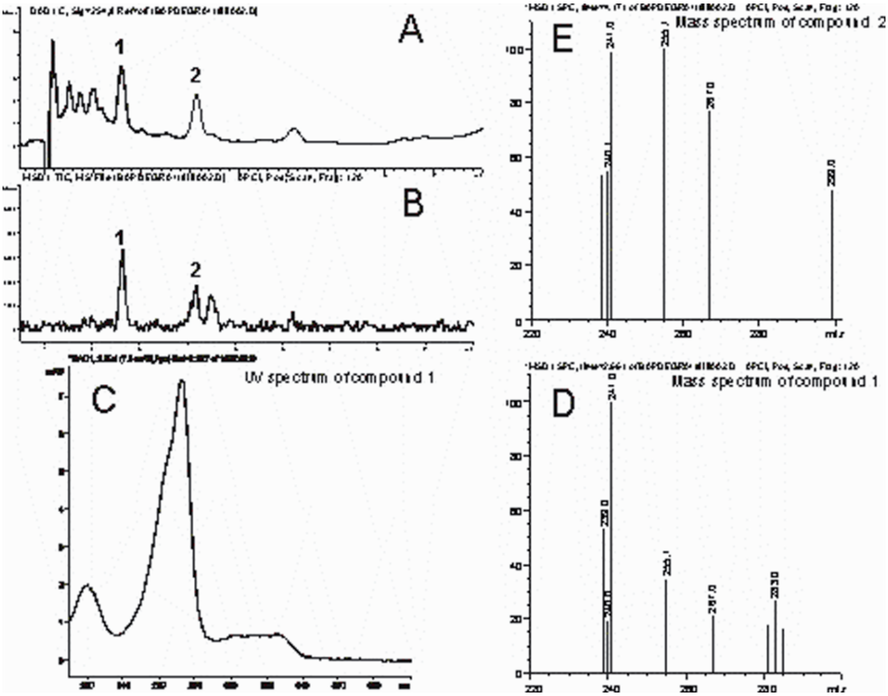

Figure 6. HPLC chromatogram of BaP solution in cyclohexane after 2 months of degradation. A: DAD detection monitored at 254 nm. B: APCI-MS detection (SCAN mode). C: UV spectrum of compound 1. D: MS spectrum of compound 1. E: MS spectrum of compound 2.

5. SIMULTANEOUS DETERMINATION OF PHENOXYACID HERBICIDES AND CHLOROPHENOLS IN SURFACE AND SEAWATER

The method for simultaneous determination of phenols and phenoxyacids based on their preconcentration using solid-phase extraction and analysis by reverse phase HPLC has been evaluated and applied for the monitoring of pollutants in the coastal waters of the Gulf of Gdansk (Baltic Sea, Poland).

Analytes were isolated and preconcentrated using the solid phase extraction process, which was performed using three different procedures. Two of them are commonly applied when either phenoxyacids or chlorophenols have to be enriched (Hewlett-Packard Application Note, Merck KGaA Application Note). The third procedure has been developed as a new method, which enables concurrent preconcentration of phenoxyacetic acids and chlorinated phenols from natural water (Kot-Wasik et al., 2004), which was unavailable using recommended as application notes methods.

The linearity ranges studied for each compound, correlation coefficient (R^2) and the detection limits of the method without a preconcentration step and after SPE preconcentration are given in Table 3.

Table 3. The linearity range of phenoxyacids and chlorophenols, correlation coefficient (R^2) and the detection limits of the simultaneous determination of analytes without preconcentration step and after SPE preconcentration.

Compound	Linearity range for analytes injected as standard solution [$x10^3$ µg/L]	R^2	Detection limit (S/N=3) Direct injection [$x10^3$ µg/L]	Linearity range for analytes preconcentrated using SPE [µg/L]	R^2	Detection limit after SPE preconcentration [µg/L]
Phenol	0.05 – 1.0	0.9996	0.02	0.5 - 5.0	0.9971	0.25
2-chlorophenol	0.05 – 1.0	0.9994	0.01	0.5 - 5.0	0.9996	0.2
2,4 – D	0.05 – 1.0	0.9993	0.02	0.5 - 5.0	0.9999	0.25
2,6-dichlorophenol	0.05 – 1.0	0.9991	0.02	0.5 - 5.0	1.0000	0.2
MCPA	0.05 – 1.0	0.9995	0.02	0.5 - 5.0	0.9970	0.25
2,4-dichlorphenol	0.05 – 1.0	0.9972	0.01	0.5 - 5.0	0.9989	0.2
dichloroprop	0.05 – 1.0	0.9991	0.03	0.5 - 5.0	0.9988	0.25
mecoprop	0.05 – 1.0	0.9991	0.03	0.5 - 5.0	0.9980	0.25
2,4,6-trichlorophenol	0.05 – 1.0	0.9997	0.02	0.5 - 5.0	0.9937	0.25
dinoseb	0.05 – 1.0	0.9984	0.01	0.5 - 5.0	0.9995	0.15
2,4,5,6-tetrachlophenol	0.05 – 1.0	0.9994	0.02	0.5 - 5.0	0.9984	0.25
PCP	0.05 – 1.0	0.9986	0.04	0.5 - 5.0	0.9950	0.25

The presented method has been applied for the determination of organic pollutants in seawater and surface water samples. The average concentrations of the chlorophenols and phenoxyacids ranged between 0.1 – 6.0 and 0.05 – 2.2 µg/L, respectively. Outstandingly high concentration of 2,4-dichlorophenol (6 µg/L) was monitored in samples collected from the Vistula River, which carry pollutants from a large part of our country. Phenoxyacids could possibly originate due to the leaching processes from surrounding agricultural areas while chlorophenols might be presented because they come from degradation processes of phenoxyacid herbicides as well as industrial activity (Merck KGaA Application Note).

6. CONFIRMATION OF THE PRESENCE OF CYANOBACTERIAL TOXINS BY LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

Most of the world's population centres rely upon surface freshwater as their primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants (like cyanotoxins) that must be identified and removed to protect public health.

Microcystins and nodularin are potent hepatotoxins produced by fresh and seawater cyanobacteria. Owing to the production of these hepatotoxins, the occurrence of aquatic blooms of cyanobacteria is of worldwide concern.

LC-ES-MS has been successfully applied for the determination of anatoxin, nodularin and microcystins in Polish lakes and seas. Figure 7 presents the example of HPLC analysis of the extract from a lake water sample allowing for the confirmation of Anatoxin A's and microcystin (MC) RR's presence.

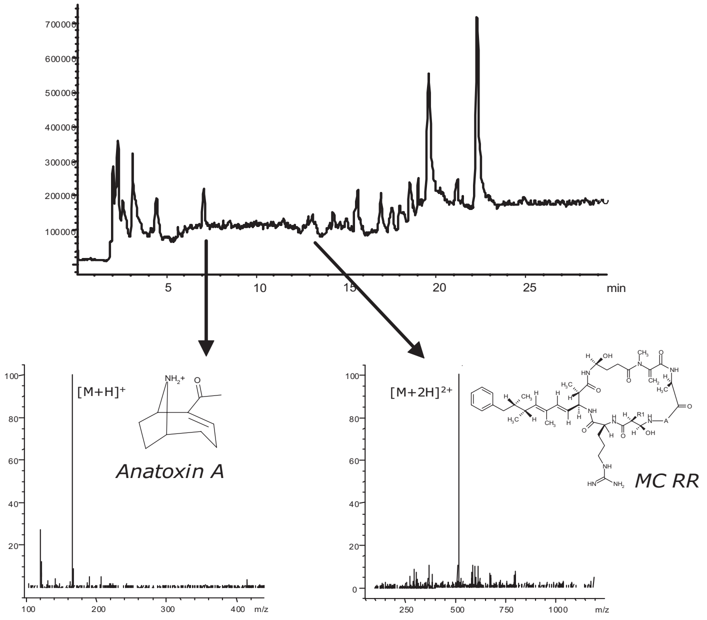

Figure 7. HPLC chromatogram and MS spectra obtained for a lake water sample allowing for the confirmation of Anatoxin A's and microcystin (MC) RR's presence.

The time of the seasonal maximum and intensity of the Nodularia bloom in the Gulf of Gdansk vary significantly from year to year. In 2001 a rapid and massive proliferation of *N. spumigena* was observed in late June - early July. The concentration of nodularin in water ranged from 90 to 18135 µg dm^{-3} and in lyophilised phytoplankton samples from 3000 to 3520 µg/g d.w. (dry weight). Such a high concentration of a toxin in the recreational waters of the Gulf of Gdansk constitutes a health risk for users of bathing areas. Other cyanobacterial toxins - microcystins and anatoxin-a - were also detected in the coastal waters of the Gulf of Gdansk (Kot-Wasik et al., 2004).

7. CONCLUSIONS

Analytical chemists involved in environmental studies are dealing with a long list of specific problems and challenges. The most important are connected with a wide spectrum of analytes and a wide range of analyte concentrations in samples of different composition and origin, therefore the development of methods enabling countercurrent determination of open range of analytes is very important. The lack of detailed information on pathways of analyte transformation and/or metabolism and emerging products of these processes force analysts to obtain more data from degradation studies.

ACKNOWLEDGEMENT

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FIBER OPTIC SYSTEM FOR WATER SPECTROSCOPY

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Abstract: An innovative series of optical fiber sensors for water monitoring, based on spectroscopic interrogation, is presented. Two types of custom-design instrumentation were developed, both making use of LED light sources and low-cost detectors to perform broadband spectral measurements in the visible spectral range. The first was designed especially to perform direct absorption spectroscopy, while the second provided turbidity measurements. Designed for water analysis and industrial process control, the proposed instruments can be used by operators with little or no technical skills.

Key words: optical fiber sensors; spectroscopy; colorimetry; online monitoring; turbidity

1. INTRODUCTION

To be able to run cost effectively, plants and processes require online monitoring by components designed to optimize operating efficiency. Optical fiber sensors, capable of performing in adverse environments, tight and/or hard-to-access spaces, and/or under real-time constraints, are increasingly replacing conventional electronic-based sensors that are not always capable of doing the job. Since they can be easily interfaced with optical data communication and secure data transmission systems, optical fiber sensors are being increasing used in industrial, automotive, avionic, military, geophysical, environmental, and biomedical applications (Culshaw and Dakin, 1988, 1989, 1996; Dakin and Culshaw, 1997; Davis et al., 1986; Grattan and Meggit, 1995, 1999a, 1999b; Krohn, 2000; Lòpez-Higuera, 2002). In addition to steadily rising quality and increasingly competitive costs, they have other welcome advantages:

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- They operate without electricity, which makes them explosion-proof and intrinsically immune to any kind of electromagnetic interference. This is a notable advantage considering the widespread use of radiating instruments.
- They are compact, lightweight, and flexible, thereby enabling them to reach what were previously inaccessible areas.
- They can withstand chemically aggressive and ionizing environments.

Spectroscopy in the visible and near infrared spectral regions is one of the most popular methods in conventional analytical chemistry (Mellon, 1950; Bauman, 1962) The intrinsic optical and mechanical characteristics of optical fibers, together with the wide availability of bright LEDs and miniaturized spectrometers, further enhance the application areas of spectroscopy, and make it possible the implementation of compact instrumentation dedicated to the monitoring of specific parameters.

This paper presents an innovative series of optical fiber sensors for water monitoring, based on spectroscopic interrogation.

- *Fiber optic systems for colorimetry*: they consist of PC-interfaced spectrophotometric units and custom software designed to fit individual sensor interrogation schemes. Their advantages include a high degree of miniaturization and compactness, flexibility, and suitability to a broad range of applications, including online water color monitoring in a water recycling plant and sea-water monitoring at the Elba Island.
- *Fiber optic systems for scattered colorimetry*: they perform multi-wavelength and multi-angle absorption spectroscopy in the visible spectral range, and made use of multivariate analysis for processing the spectral data. When similar liquids were measured, a 2D map was created. The map was populated with points, each of which represented the liquid sample with its individual and global characteristics of turbidity and also of color. Similar liquids could be mapped as clusters and, consequently, correctly assigned according to class. A successful application of scattered colorimetry was the discrimination between very similar types of water-suspended sediments.

2. FIBER OPTIC SYSTEMS FOR COLORIMETRY

The fiber optic instrumentation designed for addressing absorption spectroscopy sensors complies with the following requirements:

- High degree of compactness, i.e., the optoelectronic module plus its sources, detectors, and coupling optics fit into a compact box without requiring mechanical alignments of the optical components.

- Probe using a single optical fiber configuration, rather than bundle.
- A flexible software interface friendly enough to be used by operators with little technical background.
- Low cost optoelectronic components.

The prototype of the miniaturized fiber optic spectrophotometer for measuring the absorption spectra of liquids online is comprised of three main components:

- An optoelectronic module containing the LED sources, the spectrometer detector, and the electronic power supply.
- Custom-design fiber optic probes that allow absorption measurements of liquid samples with an optical path-length between 10 and 50 mm.
- A portable computer with custom software created in Labview® to allow dialog with the optoelectronic module and to semiautomatically acquire and store data.

Among the prototype's innovative features are compactness, cost efficiency, adaptability to specified application, ability to carry out semiautomatic and automatic measurements even under the supervision of inexperienced personnel, and low maintenance costs.

The optoelectronic module makes use of a set of LEDs to obtain a low-cost source, and a fiber optic microspectrometer by STEAG-Microparts as the detector (Boehringer Ingelheim microParts, 2005). The measuring range is from 380 to 780 nm, with 10 nm resolution, and a long-term stability of 2%. A six-LED source is provided, affording a uniform spectral intensity. The LEDs are temperature-controlled by Peltier cells to optimize source stability in terms of spectral shape and intensity. The source is made of three LEDs with broadband white emissions and three with emission peaks at 420, 500, and 640 nm. The LEDs are housed in ST-compatible receptacles for easy fiber coupling; the fiber bundle from LEDs is connected to the end in a patent-pending microoptics joint allowing connection to a single optical fiber. The highest illumination efficiency is provided by a 600 μm-core optical fiber.

LabView® software, programmed to perform automatic or semiautomatic measurements and data processing, is used for optoelectronic module management. Software management of the module enhances the instrumentation's flexibility, since several programs have been written to fit different measurement requirements (Mignani and Mencaglia, 2002; Mencaglia and Mignani, 2003).

The spectrophotometer prototype and a detail of the probe for absorption measurements of liquid samples are shown in Figures 1 and 2, respectively.

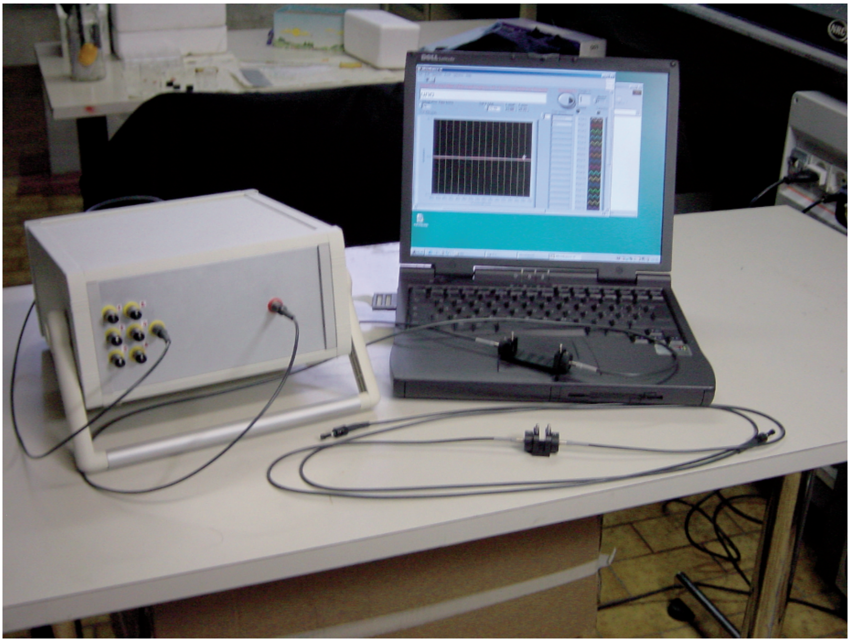

Figure 1. The prototype of the fiber optic spectrophotometer and the probes for online absorption measurements.

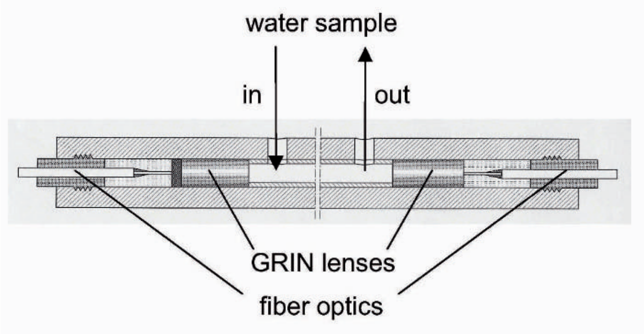

Figure 2. The fiber optic probe for measuring the absorption of liquid samples.

The prototype spectrophotometer was used in water measurements at the CONSER-IDRA water recycling plant (Prato, Italy) during October 2003. Samples (labeled A, B, and C) from three different zones were monitored (Table 1).

Table 1. The locations of the three samples analyzed from the CONSER-IDRA water recycling plant (Prato, Italy).

Sample	Location
A	Primary treatment discharge
B	Ozone treatment and recycle inlet
C	Output-recycled water

The samples were taken and analyzed over several days and at different times in order to show the absorption spectra at diverse plant operating conditions. To show the feasibility of the online measuring system, measurements were made in the recycled water inlet reservoir by placing the fiber optic probe directly inside the tank.

A set of absorption measurements are shown in Figure 3. They show that the spectrophotometer was able to follow the evolution of the samples' absorption spectra. The absorption spectra were characterized mainly by the presence of the suspended particles, which shows the same kind of scattering spectra. Nevertheless, in the case of an area of color indicating contamination, the spectra would indicate the contamination by displaying the absorption bandwidth in the vicinity of the colored area.

In addition to the absorption spectra, it is also worthwhile to calculate the integrals of the spectra that take into account the cumulative contribution of the sample color and the presence of the suspended particles, as illustrated in Figure 4. The fact that the recycled water (sample C) is of virtually constant color regardless of inlet conditions is proof that the plant is operating properly.

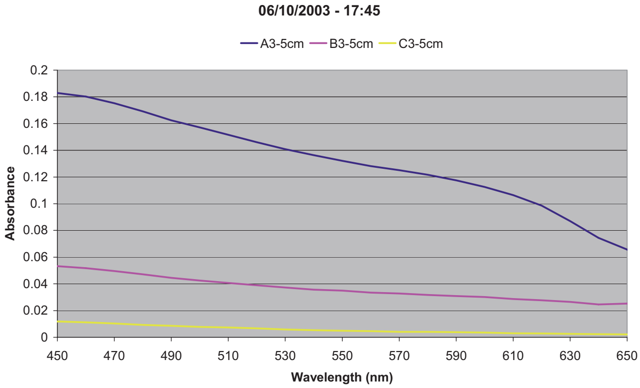

Figure 3. Absorption spectra of water samples taken from different places in the plant.

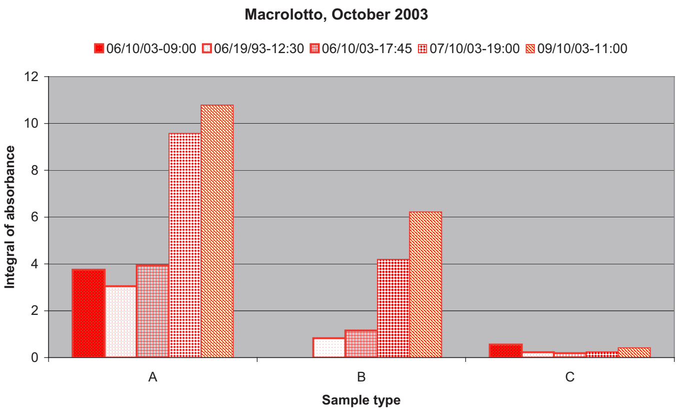

Figure 4. Integrals of the absorption spectra for the verification of the correctness of the water recycling process.

The prototype spectrophotometer was also used for sea water measurements at the Italian Elba Island to check water color at different locations and at different depths. Some spectra taken at the locations of Picco Giallo, Punta Madonna and at the harbour of Marciana Marina are shown in Figure 5. These spectra were processed by means of the Principal Component Analysis (PCA) to better cluster their similarities or differences (Cove and McNicol, 1985). The results of PCA processing are shown in Figure 6, in which the measurements result clustered according to the different locations, and also the different depths can be identified.

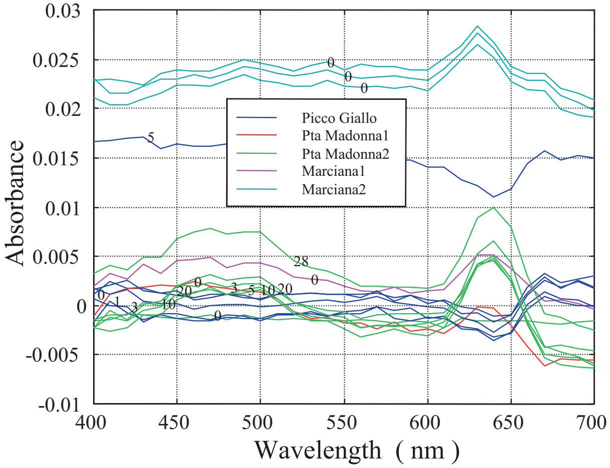

Figure 5. Absorption spectra of sea water taken at the Italian Elba Island, at different locations and depths.

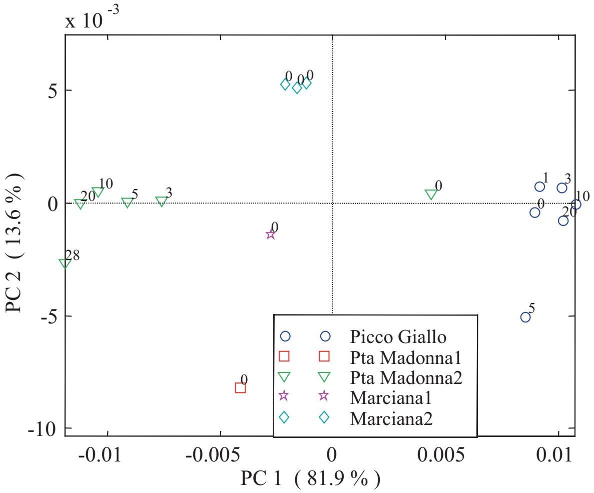

Figure 6. PCA processing of the absorption spectra capable of clustering the sea water color according to locations and depths.

3. FIBER OPTIC SYSTEMS FOR SCATTERED COLORIMETRY

Solid concentration in liquids and gases is determined by using standardised methods based on filtration and centrifuging which require the separation, drying and weighing of solids fractions. These laboratory methods are cumbersome, because they can take hours to perform and are practically impossible to automate.

In contrast, turbidity, which is an optical phenomenon due to the scattering of light by the solid particles of the suspension, is well suited for on-line and *in situ* measurements. At present, turbidity measurements are made by following the ISO7027 standard (ISO, 1984). This involves a calibrated version of the ratio between scattered and transmitted light. The ratio between the light scattered at 90 degrees and the transmitted light is measured for a formazine suspension of well-known turbidity, thus calibrating the instrument. The same quantity is then measured for an unknown suspension and is compared with that of formazine.

Although fast and practical, this method has two flaws. The first is its intrinsic ambiguity. The amount of light scattered by a suspension depends on both concentration and particle size distributions. Consequently, different concentrations of different suspensions can give the same turbidity value.

This means that it is impossible to pass from turbidity to concentration without having information on particle size distribution. Moreover, particles size itself is important information for some applications such as the testing of filter efficiency.

All this means that the turbidity standard solution must have a well-reproducible particle size distribution. This is the reason why formazine is preferred to other standards. However, formazine is the second flaw of turbidimetry, because its preparation requires the use of hydrazine sulfate, a toxic material which can be carcinogenic. Therefore, its manipulation is very dangerous. Moreover, formazine is not commercially available, and must be prepared by means of a chemical reaction, thus requiring a well-equipped laboratory (SIGRIST, 2005).

These drawbacks lead to a great demand for a new instrument that is capable of performing online turbidity monitoring and also able to discriminate among different particulates. The novel instrument presented in this paper responds to this demand. It consists of an optical fiber device that performs multi-wavelength and multi-angle scattering measurements, that is scattered colorimetry. The output is processed by means of the PCA method. This instrument has been tested by analyzing two different liquid-suspended particulates at various concentrations, thus demonstrating capability of particulate discrimination.

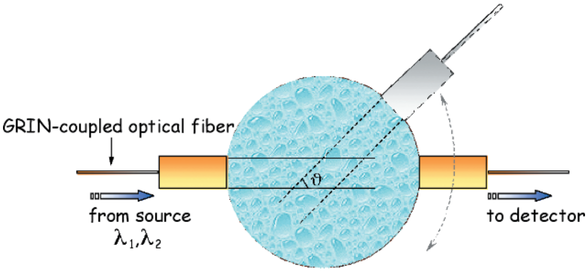

Figure 7. Working principle of scattered colorimetry.

As sketched in Figure 7, the fiber optic system for scattered colorimetry consists of a ring surrounding the test sample, along which two identical GRIN-coupled optical fibers are positioned. Fibers are connected to a light source and to a detector. The optical fiber connected to the source provides a nearly-collimated light beam. The other optical fiber, which is connected to the detector, can rotate along the ring and identifies a detecting view. A PC-interfaced rotating stage is used for motion control within a given angular range. The angle identified by the GRIN axes is the scattering angle, while the intersection between the illuminating beam and the detecting view is the area of sensitivity for multi-angular scattering measurements. The sensor output is the intensity of scattered light as a function of the scattering angle. It makes use of:

- Optical fibers 200-µm core. These fibers have large core diameter, thus enabling an efficient light coupling and the availability of high light intensity.
- NSG fiber-to-GRIN assemblies, model FCM-00F-200-0.63. These assemblies were housed in receptacles so as to avoid damage when the nephelometer was immersed in water. A glass window was placed in front of all GRINs. Silicone sealing was provided to prevent any water contact with the components inside the receptacles. The distance between the GRINs was 15 mm.
- A Micro Controle rotating stage which moves the fiber-GRIN component coupled to the detector.
- A Micro Controle TL 78 power unit and IP 28 control unit driving the stage.
- An illumination unit consisting of two commercially-available LEDs with emission in the red and IR spectral ranges and peaks at 650 nm and 850 nm, respectively. The LEDs were housed in receptacles coupled to the optical fiber by means of SMA connectors.
- A custom solid-state detector and lock-in amplifier.

This fiber optic instrument was validated by means of monodisperse LATEX beads, and demonstrated that it is able to fit the theoretical scattering patterns given by the Mie theory (Mignani et al., 2001).

The ability of the fiber optic instrument for scattered colorimetry to discriminate among different water-suspended particulates and to measure their concentration was tested by using two kinds of dust. These were obtained by filtering the basic *Arizona Fine Test Dust* (ISO12103), which is a standard material for filter testing. They are named Fine (F) and Coarse (C), and consist of particles widely polydisperse and with overlapping size distribution. Figure 8 shows the Probability Density Function of these particulates (ISO, 1997).

Scattering measurements of F and C particulates, at 650 and 850 nm, respectively were taken. Each suspension was measured at five concentrations in the 10-60 mg/L range. All scattering measurements were normalized to light intensity transmitted at 0°.

PCA was used to process the scattering/spectral data, in order to create a 2D map that clusters the different types of suspensions, thus providing their discrimination, as shown in Figure 9. The sign of the second principal component indicates the type of particulate, since the F and C particulates are identified by means of a negative or positive sign, respectively. The reliability of this map as far as particle characterization is concerned is also shown by the trend of the behavior of the principal components, which increase with concentration, converging towards zero for null concentration.

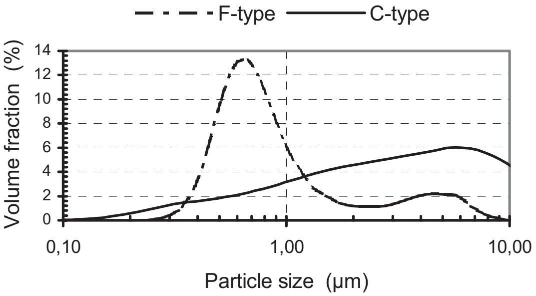

Figure 8. Probability Density Functions of F- and C-type Arizona Test Dust.

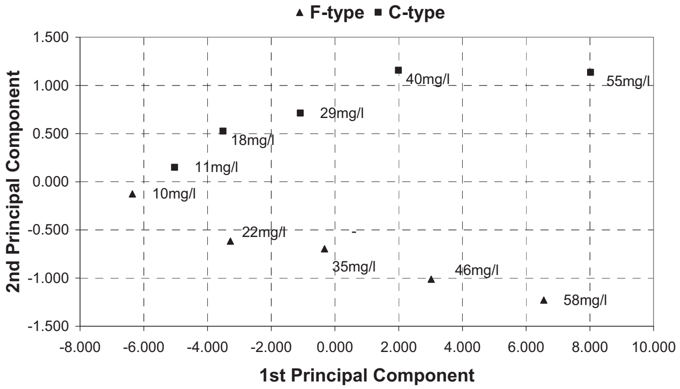

Figure 9. Discrimination of F and C particulates by means of the PCA method for scattering/spectral data processing.

4. CONCLUSIONS

The fiber optic spectrophotometers here presented comprise PC-interfaced electrooptic units programmed to fit individual sensor interrogation schemes. Their advantages include a high degree of miniaturization and compactness, flexibility, and suitability to a broad range of applications. Thanks to the availability of several functional materials for selective molecular recognition that exhibit optical absorption in the visible spectral range modulated by interaction with the analyte, the instrumentation is potentially suitable for a wide range of sensors and for olfactory perception in process control, safety, and environmental applications. Designed for the color analysis of liquids and surfaces in industrial process control, water analysis, and food processing control, the proposed spectrophotometers can be used in other colorimetric applications by operators with little or no technical skills. The presence of colorimetric passive samplers acting as dosimeters for gaseous pollutants and acids enables the instrumentation to act as an in-situ spectrophotometric laboratory, i.e. a totally autonomous nonstop monitor of passive samplers in a host of industrial and environmental applications.

The fiber optic instrument for scattered colorimetry, as it is able to provide multi-wavelength and multi-angular scattering measurements, demonstrated an ability to measure simultaneously the concentration of a suspension and its type, whether F or C, thus removing the intrinsic ambiguity of conventional turbidimeters. The use of optical fiber technology made it possible not only to obtain a compact and versatile device for online monitoring, but also an instrument capable of performing very accurate scattering measurements as far as the angular resolution of these measurements is concerned. The effectiveness of PCA data processing was enhanced by this optimized accuracy, which enabled discrimination between very similar types of particulates. For these reasons, scattered colorimetry has the potential to discriminate also mixtures of suspensions.

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PREDICTING METAL UPTAKE BY PLANTS USING THE DGT TECHNIQUE

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Abstract: Measurements in soils of the effective metal concentration using DGT (diffusive gradients in thin-films) generally correlate very well with the concentrations of metals in plants grown in the same soil. While the goodness of fit varies, depending on the metal, plant species and soil type, it is generally better than the fit to other soil measurements. A dynamic model that has been used to emulate the DGT-soil system has been shown to have many features in common with dynamic models of plant uptake. The diffusion layer of DGT controls the uptake flux in a range that is compatible with uptake fluxes of plants. DGT does not take into account metal supplied by mass flow, but with the exception of polluted sandy soils where there is a small reservoir of metals in the solid phase, this does not usually contribute significantly to the uptake. DGT mimics directly the supply from solution and the kinetically limited release from the solid phase that accompanies this depletion in solution. The good correlations with plant uptake and the modeled depletion at the DGT and plant surface shows that the kinetics of supply are important, suggesting that the free ion activity model is not applicable. DGT can be used directly to obtain the rate constant of supply from solid phase to solution and the size of the solid phase reservoir of labile metals. These are both important terms in any model of the dynamics of metal uptake by plants.

Key words: DGT; soils; plants; metals; DIFS; bioavailability; dynamics

1. INTRODUCTION

Scientists have striven for years to predict the concentration of metal in plants from some measure of the metal in the soil in which they have grown (Rieuwerts et al., 1998; Brummer, 1986). Whatever measure of metal in the

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soil is chosen, be it free ion activity, total concentration in soil solution or metals extracted with various reagents, successful prediction implies a certain mechanism of uptake (McLaughlin et al., 1998). For free ion activity to be predictive, uptake by the plant must be so slow that it does not induce depletion in the concentration of metal in the soil solution immediately adjacent to the plant (Campbell et al., 1995). A good correlation with the total metal in soil solution implies a fast uptake by the plant, so that the concentration of the species actually taken up by the plant (often assumed to be the free ion), is depleted in the soil solution adjacent to the plant. This provides the opportunity for dissociation of metal complexes in solution. All complexes must dissociate rapidly (labile) if they are to be measured. There must be no release of metal from the solid phase because this would vary with pH and other soil characteristics, causing the correlation with metal in soil solution to break down. The third possibility, where metal extracted from the solid phase correlates well with metal in the plant, can be met if metal can be released from the solid phase in response to its removal from solution by the plant. As concentrations of metal in solid phase pools are usually much greater than in solution, the supply from this source can become dominant. The various extraction schemes identify different pools of metal in the solid phase, with the presumption that metal is supplied exclusively from this pool that has been defined by the chemical reagent and conditions used.

For correct prediction by any of the three types of measurements given above, of metal taken up by plants, the release of metal within the soil in response to plant uptake must be restricted to a particular mechanism. However, texts that have considered the dynamics of plant-soil interactions (Barber, 1995; Marschner, 1995) have demonstrated that the processes mentioned above are not isolated. Apart from the exceptional case of control by the activity of the free ion (very slow uptake), supply is likely to occur simultaneously from complexes in solution and from the solid phase. The dynamics of these supply processes, as well as the total pool sizes, are likely to regulate the amount of metal reaching the plant.

An alternative approach to measuring pseudo-equilibrium chemical characteristics (free ion activity, total concentration in soil solution, an extractable pool), is to mimic the dynamic action of the plant. The metal that is released will then automatically reflect the different supply terms. This is the approach of the technique of DGT (diffusive gradients in thin-films) (Davison and Zhang, 1994, Zhang et al., 1998, Zhang et al., 2001). DGT uses a diffusion layer of hydrogel overlying a chelating resin to remove metal from soil solution at a controlled rate. The metal that is released over a period of time is measured by analysing the Chelex layer. If the rate of removal of metal emulates the rate of removal of metal by the plant, the soil is similarly perturbed in both cases. Therefore, the supply from dissociation of complexes and release from the solid phase will be the same for both DGT and the plant. In this case the amount of metal accumulated by DGT in a given time should correlate well with the amount taken up by the plant (Lehto et al., 2006). The first comparisons of metals taken up by DGT and by plants showed exceptionally good agreement (Davison et al., 2000; Zhang et al., 2001), but as the available data sets have increased, exceptions have emerged (Nolan et al., 2005). This paper reviews the understanding of the DGT-soil system in the context of its mimicry of the plant-soil systems and examines the available evidence for the capability of DGT as a tool for predicting metal concentrations in plants.

2. DGT-SOIL INTERACTIONS

The basis of DGT has been described in detail (Zhang et al., 1995; Davison et al., 2000). For measurement of cationic metals, it comprises a plastic holder that contains a binding layer of Chelex resin embedded in a layer of gel (Figure 1). This resin-gel is overlain by a layer of hydrogel and a filter, through which metal can freely diffuse. Collectively the hydrogel and filter membrane are known as the diffusion layer.

The effect of DGT on a soil is very simple. It removes metal from soil solution by accumulating it on the Chelex resin. The metal must first diffuse through the filter membrane and the layer of gel. This diffusion layer sets a maximum possible flux to the DGT devices. To explain this further, the possible response of the soil to the perturbation imposed by DGT will be considered. A key point is that DGT continuously removes and accumulates metal and so supply processes from the soil must be considered dynamically. In response to the removal, the concentration of metal in soil solution adjacent to the device is lowered. Metal will therefore dissociate from complexes in solution to try to re-establish the equilibrium state that has been perturbed. Consequently all species that are labile, that is able to dissociate sufficiently rapidly to contribute to the flux to the device, will be measured (Zhang and Davison, 2000). However, there is also a dynamic exchange of metal between solution and solid phase. Local depletion of metal in soil solution will induce release of metal from the solid phase at the site of depletion. As for release from complexes in solution, the extent of this supply of metal from the solid phase will depend on its rate of release.

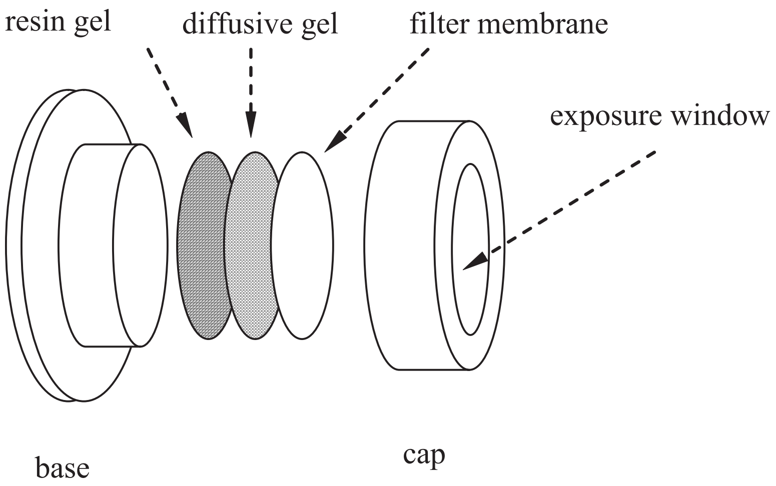

Figure 1. Schematic representation of a DGT device.

Figure 2 illustrates how the extent of depletion in soil solution depends on the rate of release of metal from the solid phase. When the rate of release is very fast, the supply from the large pool of metal in the solid phase effectively buffers the metal concentration in soil solution so that very little depletion occurs. The rate of metal uptake is then controlled by the DGT device. It is governed by the thickness of the diffusion layer. Depletion of metal in solution will eventually occur, even when there is fast release, because the metal in the solid phase adjacent to the device will gradually be used up. If the rate of release from the solid phase is very slow, there will be little supply from this source, and metal supply will depend on diffusion of labile species in solution. The metal in solution adjacent to the device is quickly depleted with time, causing the flux of metal to the DGT device to decline quite rapidly. The intermediate case occurs when metal is released from the solid phase at an appreciable rate, but it is insufficient to sustain fully the concentration in solution adjacent to the device.

DGT measures directly the amount of metal that accumulates in a given time. This can be used to calculate the mean concentration at the surface of the device during the deployment time, often known as C_{DGT}.

The above processes can be quantified by modeling simultaneously and dynamically the supply of metal by diffusion and its release from the solid phase (Harper et al., 1998). The approach that has been used so far is to assume a pool of labile metal in the solid phase that is related to the

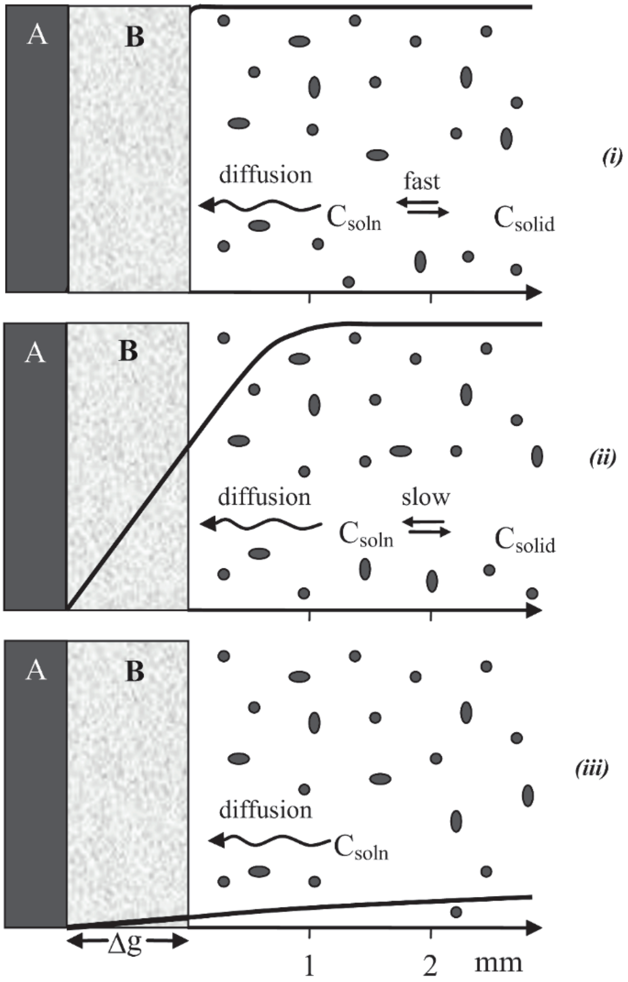

Figure 2. Schematic representation of the concentration of metal in solution in DGT and the adjacent soil after 24 hours, for three cases: (i), very fast release from solid phase; (ii), fairly slow release; (iii), no release. Layer A is the resin gel and layer B is the diffusion layer.

concentration of metal in the solution phase by a partition coefficient, K_{dl}. The uptake of metal by the resin is supplied by diffusion and release from the solid phase, which is governed by first order kinetics. A one dimensional version of this model, know as DIFS (DGT-induced fluxes in soils) has been available for several years (Harper et al., 2000). A full two dimensional version, which more accurately reflects the diffusional supply, has been recently developed (Sochaczewski et al., 2005).

The dependence of the ratio of C_{DGT} to the concentration in soil solution on the DGT deployment time has been measured for a range of metals and soils (Ernstberger et al., 2002, 2005). The results were fitted very well by the DIFS model, which provides confidence in the model formulation and our interpretation of the metal interactions occurring in the soil. Values of the pool size of labile metal and the rate constants for release from the solid phase were obtained from the model fits. These showed that while the supply of Ni may be kinetically limited, it is unlikely that this will be the case for Cd and Zn. The kinetics of release of Cd and Zn from the solid phase were measured by DGT in a wider range of soils (Zhang et al., 2005). This work showed that the rate of release of both metals was too fast to measure in clay soils. The slowest rates of release were observed in silty or sandy soils with low to intermediate pH (4.84 - 5.66).

3. PLANT-SOIL INTERACTIONS

Like DGT, plants accumulate metals by removing them from soil. The perturbation of the soil is similar if the rate of removal by the plant and DGT is similar. Lehto et al. (2006) have used models of uptake of metals by plants and DGT to show that fluxes to plants and DGT are generally similar for typical values of diffusion layer thicknesses used in DGT devices. Hyperaccumulator plants will tend to have slightly higher uptake fluxes than DGT.

Processes that are not mimicked by DGT can also affect the supply of metals to plants, including convective transport, the root encountering fresh surfaces as it grows through the soil and the influence of root exudates and microenvironments. For DGT to be effective in predicting plant uptake, the contribution from each of these processes must be small compared to supply by diffusion and associated release from the solid phase. According to the accepted ranges of mass flow (Barber, 1995), modeling indicates that supply by convection is usually negligible compared to diffusive supply, especially when release from the solid phase is also considered (Lehto et al., 2006). It is more difficult to model the other terms that are not mimicked by DGT. When good relationships between metal accumulated by DGT and plants are obtained, it is reasonable to suggest that these terms do not contribute appreciably to metal supply to the plant. Their possible significant contribution to supply may be one of the reasons for such relationships breaking down.

4. EXPERIMENTAL EVIDENCE

In the first comparison of DGT measurements with metal taken up by a plant (*Lepidium sativum L.*), DGT was deployed and plants were grown in the same soil, but at different moisture contents, representing 50 to 90% of the maximum water holding capacity (Davison et al., 2000). The concentration of Cd, Co, Cu, Ni, Pb and Zn in the plant herbage and the flux of metals to the DGT device both increased systematically with moisture content, while the concentration of the metals in soil solution declined. As only the water content in the same soil was varied, the concentration of metals in soil solution can be expected to be proportional to the free ion activity. These results therefore clearly showed that neither the free ion activity of metal nor the concentration of metal in soil solution could predict the metal taken up by the plant. The fact that DGT could predict plant uptake was attributed to supply by mass transport and associated release from the solid phase being dominant and their dependency on moisture content being similar for both DGT and the plant.

In the second comparison an indicator species for Cu (*Lepidium heterophyllum* Banth.) was grown in 29 different soils with a range of Cu concentrations. Copper was measured by DGT, in soil solution, as free ion activity and by EDTA extraction (Zhang et al., 2001). The DIFS model was used to convert the DGT measured concentration, C_{DGT}, to an effective concentration, C_E, which includes the Cu in soil solution plus an additional term representing Cu supplied from the solid phase. An exceptionally good correlation was obtained between the Cu concentration in the herbage and C_E, whether plotted on a logarithmic ($r^2 = 0.95$) or linear ($r^2 = 0.98$) scale. Markedly inferior correlations were obtained for the free ion activity of Cu and the EDTA extractable Cu, but a reasonable logarithmic relationship was obtained for Cu in soil solution. The good correlation for C_E, as measured by DGT, for this very wide range of soils, suggested that the processes mimicked by DGT, namely supply of Cu by diffusion and release from the solid phase, dominated supply of metal to the plant for a wide range of conditions.

This type of experiment, where the concentration of metal in herbage is correlated with metal measured by DGT, has been replicated by several laboratories using a wide variety of soils, metals and plants. *Elsholtzia splendens* and *Silene vulgaris* were grown on substantially the same set of contaminated soils (Song et al., 2004). Reasonable correlations were obtained between the concentration of Cu in the herbage and C_E, measured by DGT. Although these relationships were not significantly better than the corresponding ones for extractable Cu (1M NH_4NO_3) or soil solution Cu, they were better than those for total soil Cu, EDTA extractable Cu or free Cu^{2+} activity.

Lepidium sativum was grown in four different soils collected close to galvanized pylons or busy roads and variously contaminated with Zn (Zhang et al., 2004). The same soils with low background Zn were amended with Zn to similar concentrations to the contaminated samples. Zn in the herbage was more closely correlated with C_E (for field contaminated and amended soils) than with Zn in soil solution or total soil Zn. Zn in the plant only increased systematically with C_E above a threshold concentration. This observation was attributed to homeostatic processes controlling internal Zn concentrations at optimal levels until supply from the soil, as indicated by C_E, is large enough to override this control. DGT was used to show that the rate of release of Zn from the solid phase was faster in amended soils.

Koster et al. (2005) measured Zn in grass, lettuce and lupine grown in 28 soils with various textures and amended amounts of Zn. Good correlations were found between Zn concentrations in grass and lettuce and C_E, Zn extracted with $CaCl_2$ and soil solution Zn, but the correlation between Zn in lupine and C_E was not significant.

Nowack et al. (2004) found good relationships (non-linear) between Cu and Zn in the herbage of *Lolium perenne* (ryegrass) and C_E measured by DGT. The source of metal contamination was filter dust from a brass smelter. The same relationships were observed for soils freshly contaminated and those previously contaminated in the field, whether or not the soils were homogenized, showing the robust nature of the predictive capability of DGT. The non-linear relationship was attributed to saturation type behaviour, which is appropriate for a metal excluder plant, such as ryegrass, where there is limited translocation between roots and shoots. These workers also measured Cu and Zn uptake by ryegrass and DGT directly in the field. Some deviations from the relationship obtained in the laboratory were attributed to local variations in microsite conditions.

The toxicity of Zn to *Sorghum vulgare* grown in sand amended with $ZnSO_4$ and mine wastes, assessed as 90% of the control yield, was well predicted by both DGT measured Zn and Zn extracted using $CaCl_2$ (Sonmez and Pierzynski, 2005).

The toxicity responses of barley (root elongation) and tomato (shoot growth) to Cu additions were performed on 18 European soils representing a wide range of soil types and soil properties (Zhao et al., 2005). The relationships between the concentrations of Cu in soil solution, free Cu^{2+} activities or DGT measurements and the toxicity response were investigated. The DGT measurement was found to narrow the inter-soil variation in EC_{50} considerably, and to be a better predictor of plant Cu concentrations than either soil solution Cu or free Cu^{2+} activity.

The widest comparison of different measurements of metals in soils, which includes C_E and metal concentrations in plants, was undertaken using wheat (*Triticum aestivum L.*) grown in 13 metal contaminated soils (Nolan et al., 2005). In addition to C_E, Zn, Cd, Pb and Cu were measured as total concentrations, total dissolved metal, free metal ion activities in soil solution, $CaCl_2$ extractable metal and E values (exchangeable labile metal) measured by isotopic dilution. Zn concentrations in wheat correlated best with C_E, Cd with C_E and $CaCl_2$ extractable equally, and Pb with C_E and total dissolved Pb equally. Surprisingly the total concentration of Cu gave the best correlation.

This growing data base on DGT suggests that it is generally a good predictor of metals in plants, but it is not infallible. For Zn and Cd, the concentration of metal extracted by $CaCl_2$ often appears to match the predictive capability of DGT. The kinetically available pool of labile solid-phase metal, which is involved in both the DGT measurement and the $CaCl_2$ extraction, appears to contribute significantly to the metal supplied to the plants. This finding agrees with DGT studies of the kinetics of release of Zn and Cd from the solid phase, which indicates generally fast supply (Ernstberger et al., 2002 and 2005, Zhang et al., 2005). It might be worth looking at those soils which caused data to deviate from good correlations to see whether they fall into a sand/silt, low pH category, where the supply from the solid phase could be kinetically limited.

The equal performance of C_E and the concentration of metal in soil solution for predicting the behaviour of Pb is so far restricted to one data set. However, it is consistent with the known strong sorptive properties of Pb, which are unlikely to result in substantial and fast supply from the solid phase. Without this supply, metal accumulated by both plants and DGT are likely to be related directly to the total labile species in solution, which usually approximates to the total concentration in solution.

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ON CONCEPTUAL AND NUMERICAL MODELING OF FLOW AND TRANSPORT IN GROUNDWATER WITH THE AID OF TRACERS: A CASE STUDY

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Abstract: Transport models based on calibrated flow models may often yield wrong predictions unless recalibrated with the aid of environmental tracers because migration velocity depends on the ratio of hydraulic conductivity to porosity whereas flow rates depend on transmissivity. An environmental tracer (SF_6) was used to improve calibration of transport and flow models in a sandy aquifer. Simulated and measured ^3H contents showed that further improvement of the conceptual model and recalibration of flow and migration models are needed for some parts of the aquifer. Residence time distributions obtained from lumped-parameter and numerical models are shown to serve for a better understanding of the groundwater system.

Key words: conceptual model; flow and migration models; water age; SF; tritium

1. INTRODUCTION

Environmental tracers are helpful in construction of conceptual models and for calibration or validation of flow and transport models. However, in field practice, tracer or pollutant data very often do not agree with the numerical flow and transport models. Flow models are commonly calibrated

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by fitting of transmissivity values (T = K×m, where m is the aquifer thickness and K is its hydraulic conductivity). Therefore, a model calibrated hydrodynamically does not yield proper values of K, whereas migration velocity of solutes depends on the K/n_e ratio, where n_e is the effective porosity, i.e., that one in which the water movement takes place (Figure 1). It is evident that a model properly calibrated hydrodynamically may disagree with tracer data, and, if not recalibrated, will yield wrong prediction of pollutant migration.

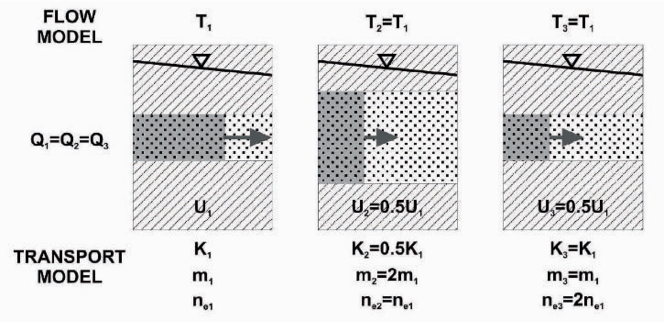

Figure 1. Parameters influencing the calibration process of flow and transport models.

By neglecting all possible inaccuracies of the tracer method (e.g. the influence of matrix diffusion for all tracers and carbon hydrochemistry for the ^{14}C method), recalibration of the transport and flow models with the aid of environmental tracers, and/or with available pollutant distributions, should lead to models satisfactorily describing the investigated systems. Unfortunately, there are limitations inherently related to tracer methods, which are explained by the following imaginary model shown in Figure 2.

Consider steady state flow from the recharge area to an observation well (no pumping) at some distance. The uppermost rectangle in Figure 2 shows that tracer ages and hydrodynamic ages are the same, e.g. 100 years. Next, the observation well is changed to the pumping well and due to a new hydraulic gradient, the flow rate increased by a factor of 2, and a new hydrodynamic steady state is formed after, say, 10 years (second rectangular). In such a situation, the hydrodynamic age is 50 years, but water that reaches the well was recharged 90 years ago, and its tracer age is 90 years. Hydrodynamic is in a steady state, but tracer ages are not in a steady state. After next 40 years of pumping (total 50 years), the tracer ages will reach a new steady state, in agreement with the hydrodynamic age (third rectangular). In other words, the age by the tracer method yielded the mean historical value, whereas the numerical model, though, in principle, can describe any unsteady situation in the past, in practice is unable to do it properly due to the lack of adequate information on boundary conditions (e.g. hydraulic gradient, recharge conditions).

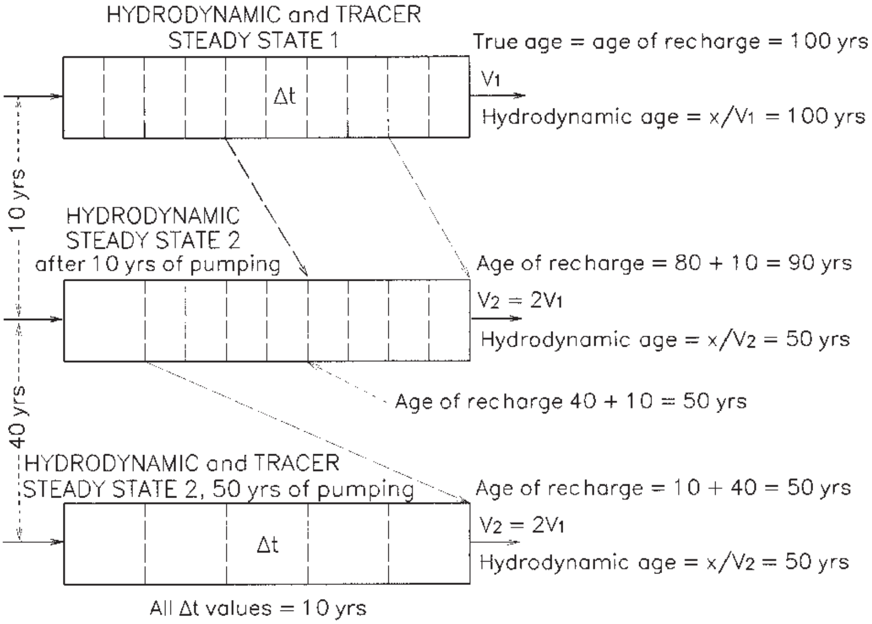

Figure 2. Imaginary piston flow model showing possible large differences between hydrodynamic and tracer ages (see text for explanation).

Therefore, in some cases, discrepancies between tracer data and simulations by numerical transport models are unavoidable. For instance, it is even possible to imagine a system with the hydrodynamic ages of 1 ka or less, and tracer ages of 10 ka, if for the whole Holocene the hydraulic gradient was much lower than during the human activity in last several hundred years.

For further considerations, the model of Figure 2 was not taken into account; however, it probably explains some discrepancies between the tracer data and simulated values still remaining after recalibration.

According to generally accepted knowledge and UE directives (Guidance Document, 2003), modeling of groundwater systems is of importance for a proper monitoring and management of groundwater bodies. Within the EU Fifth Framework Project (Project EVK1-CT1999-0006: "Natural Baseline Quality in European Aquifers: a Basis for Aquifer Management") two aquifers were investigated in southern Poland for obtaining baseline chemical data and for developing a proper modeling methodology, including Tertiary sands and sandstones of deltaic origin. Hydrochemical data of that aquifer were briefly presented by Dulinski et al. (2002), whereas aspects related to tracers and modeling by Zuber et al. (2005). Within this work some other problems related to the latter aspects are discussed in more detail.

2. STUDY AREA

A simplified hydrologic map of the aquifer is shown in Figure 3, whereas the cross-section is presented in Figure 4. The recharge takes place at the outcrops of Tertiary sands in the elevated southern part of about 2-km width, and by downward seepage through Quaternary loess and loam sediments. General flow direction is from the south to the Vistula valley in the north-east, where natural drainage takes place by upward seepage through Badenian clays. Intensive withdrawal created local depression cones with additional recharge by enhanced downward seepage. Hydraulic conductivity values of the Tertiary sediments decrease in the direction of flow from 8.8×10^{-5} to 2.3×10^{-6} m/s (geometric mean of 1.5×10^{-5} m/s). Hydraulic conductivities of low permeability sediments do not show any directional trend, and are from 1.2×10^{-9} to 3.7×10^{-9} m/s. For Quaternary sands, 0.30 porosity was assumed, and for Tertiary sands and clays it is known to be 0.25 ± 0.075.

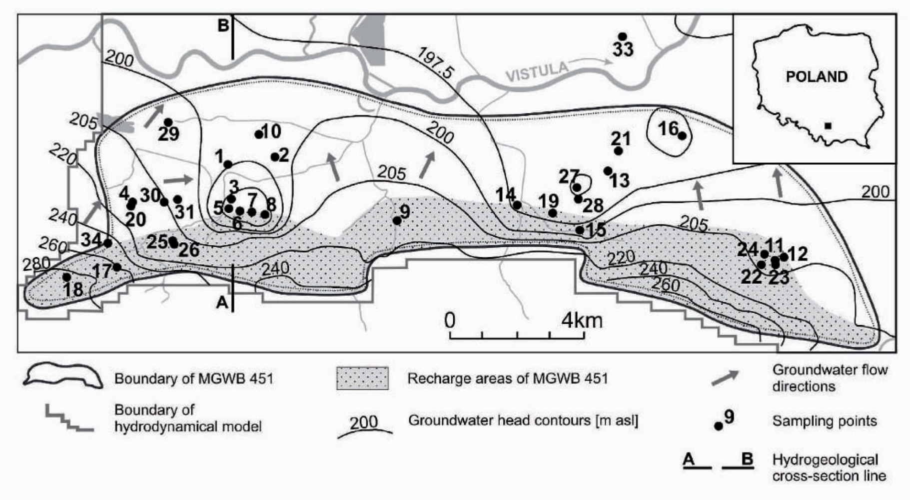

Figure 3. Simplified hydrogeological map of the investigated area.

Mean atmospheric precipitation, evapotranspiration and infiltration rates are 760, 500 and 140 mm/a, respectively. Safe yield are estimated at 40,000

m^3/d, with typical well rates of 96 to 4,800 m^3/d (Kleczkowski et al., 1990). The presence of tritium, SF_6 and freons indicates the domination of modern waters in the recharge areas and in nearby covered areas. Older Holocene waters occur in more distant areas as indicated by the lack of tritium and SF_6, and moderate ^{14}C contents. In the most distant wells, glacial waters occur as deduced from their $\delta^{18}O$, δ^2H, ^{14}C, Ne and Ar values.

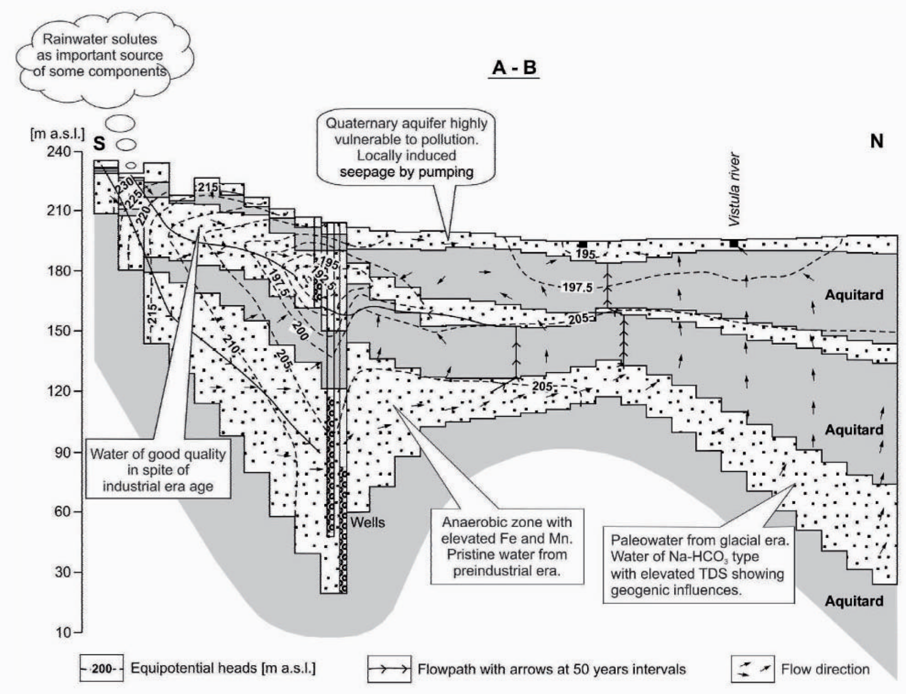

Figure 4. Simplified cross-section A-B (see Figure 3) and the conceptual model structure.

3. NUMERICAL FLOW AND TRANSPORT MODELS

Numerical modeling was performed for variable migration and steady hydrodynamic field. The model area was divided into 250m×250m blocks in 5 layers (Quaternary sands, Badenian loams, upper sands and sandstones, clays, lower sands and sandstones) underlain by impermeable sediments. Steady flow conditions were assumed because withdrawal rates have been nearly constant in the several last decades. Withdrawal wells and infiltration from precipitation were regarded as boundary conditions of the second type (specified flow boundaries). Spatial variations of the infiltration rate were within 70 to 230 mm/a. Head-dependent flow boundaries (type 3) were used at the south and north borders and at rivers. No-flow boundary conditions were used at the west and east borders.

Simulations performed with the aid of Visual MODFLOW, MODPATH and MT3D (Waterloo Hydrogeologic, 2003). For SF_6, the migration time through the unsaturated zone was neglected, and the measured values were corrected for the air excess, altitude and temperature of recharge (Zuber et al., 2005). For tritium, the travel times through the unsaturated zone were estimated for particular blocks according to the infiltration rate, thickness (1-30 m) and moisture content, with uncertainty of several years. The dispersivity (α_L) was taken as equal to 50 m.

Simulated SF_6 contents calculated with MT3D for hydrodynamically calibrated flow model yielded in most cases large discrepancies with the measured values (Figure 5). Recalibration, i.e., change of parameters to obtain better fits both for the hydrodynamic field and the SF_6, yielded an improved model. For instance, for wells 9 and 15, the initial contribution from streams had appeared to be initially overestimated as indicated by low tracer contents. For wells 5 and 6, increased values of simulated SF_6 contents are justified by larger recharge area as discovered during the construction of a highway. Unfortunately, it was not possible to obtain a good fit for all wells (Figure 5).

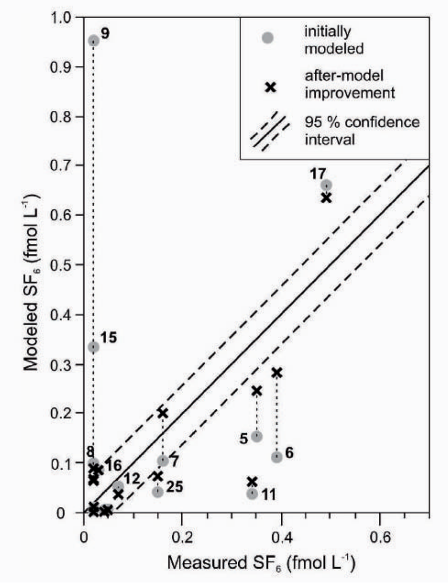

Figure 5. Comparison of simulated and measured SF_6 contents.

Significant differences between simulated and measured SF_6 remained in well 11, whereas the presence of SF_6 in well 25 is of doubtful origin because the lack of tritium suggest the presence of pre-modern water (tritium was measured 3 times in the period of 2000-03 and SF_6 twice in October 2002 and May 2003, Zuber et al., 2005).

The recalibrated model was next used to simulate space and time distributions of tritium. For some wells, the agreement with measured values was very good or reasonably good (wells 5-8 in Figure 6). For others, especially in the east part of the aquifer, where the geology is poorly known, no satisfactory agreement was obtained. For instance, the measured values in well 11 have a rising tendency whereas those simulated decrease in time. Discrepancies are also observed for several other wells, the largest in well 25, mentioned earlier. That well is situated in the recharge area and simulated content for 2003 is about 10 T.U., whereas measured values are still at zero content within the uncertainty (about 0.3 T.U.). Most probably, in that area, a large and deep erosion structure exists, which requires a change in the conceptual model, if confirmed by geophysical investigations.

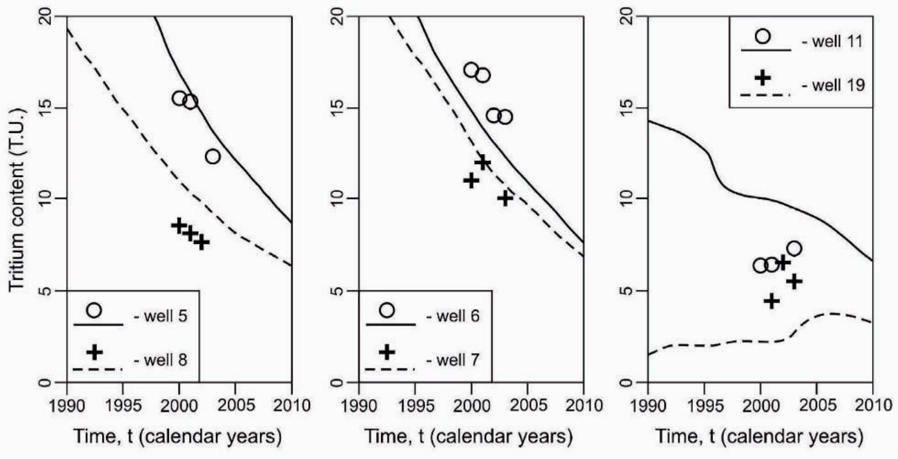

Figure 6. Simulated and measured tritium contents in selected wells.

For wells with records of tritium data, their interpretation was performed with the aid of lumped-parameter models to obtain mean ages and residence time distributions (RTD) of flows (Zuber et al., 2005). The same RTD functions were obtained for any chosen point in the aquifer by simulating migration (with the aid of MT3D code) of a conservative tracer injected instantaneously on the whole recharge area. Such simulations by definition yield the RTD functions, which, unfortunately, are seldom used though they permit a better understanding of the aquifer response to any real or potential pollution. For instance, shown in Figure 7 bimodal distributions served for identification of flow contributions from streams and rivers (early peaks), or flows resulting from upward seepage from lower layer (very late peaks).

For some wells, RTD functions found from the lumped-parameter models agree reasonably with the simulated functions as shown for well 7 in Figure 8. Lumped-parameter models yield the inverse solution (parameters found by fitting to the tritium data) whereas earlier calibrated numerical model yields direct solutions.

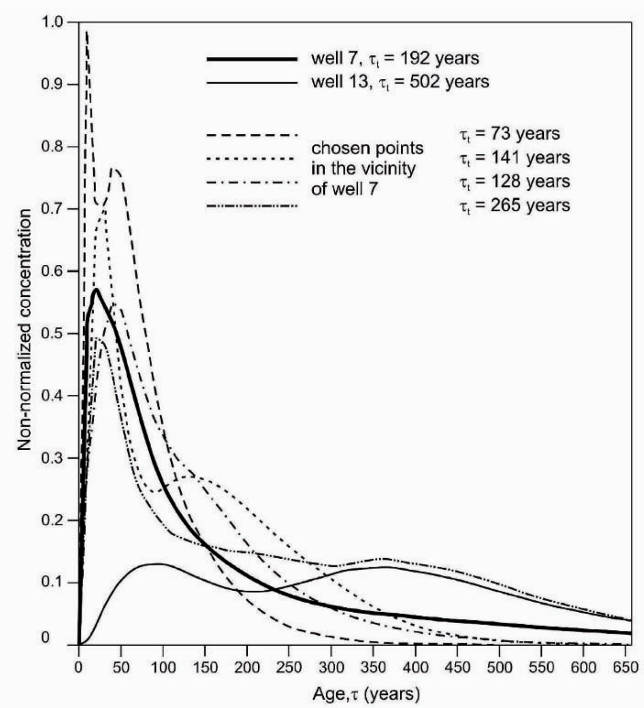

Figure 7. RTD functions for the upper aquifer layer obtained with the aid of MT3D. Vertical scale shows the probability of the occurrence of water with a given age.

Notice that concentration scale for lumped-parameter models can be normalized as the whole tracer theoretically reaches the well (output of the system). In numerical models only a very small fraction of the injected tracer reaches the observation well (the whole drainage are represents the outlet of the system). The RTD functions exhibit very large differences in flow times even for wells in the recharge areas (a reflection is suggested to those who apply the piston flow approximation for transient tracers). In cases of large dispersion, lumped-parameter models yield similar RTD functions for a number of different pair values of the mean age (τ_t) and the dispersion parameter (P_D), because then an interplay of parameters occur.

In the discussed case, a large value of the dispersion parameter (P_D) is a result of a very different flow paths and has nothing to do with the common dispersion in an unidimensional flow where $P_D = D/vx = \alpha_L/x$, with D being the dispersion coefficient, v the mean velocity, x the flow distance, and α_L the axial dispersivity.

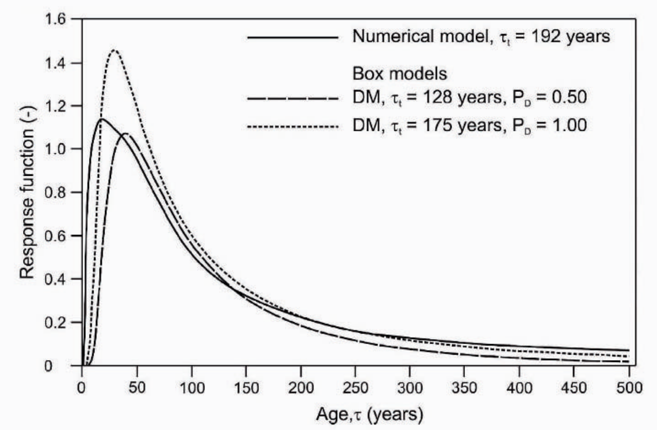

Figure 8. RTD functions for well 7 obtained by numerical simulation and lumped-parameter models (Maloszewski and Zuber, 1996). The vertical scale is not normalized for the numerical model.

4. CONCLUSIONS

In general, uncertainties of numerical modeling of migration in groundwaters mainly result from: (a) imprecise conceptual model; (b) inadequate calibration of flow model, which in principle is not sufficient for migration model; (c) inadequate structure of the numerical model (e.g. dimensions of blocks or time steps); (d) uncertainty of exploitation history (more generally the lack of knowledge on the past boundary conditions); and (e) poor knowledge of the space distribution of travel times through the unsaturated zone.

Within this study such environmental tracers as tritium and SF_6 were shown to be very useful for a better calibration of flow and transport models, especially to reduce the uncertainty listed above under “b”. They also are helpful in a better understanding of the system, which may lead to an improvement of the conceptual model.

For the investigated aquifer, discrepancies between the observed data and simulated values, which were not reduced in the recalibration process, show that a better geological knowledge is required, particularly in the east part in order to improve the conceptual model. According to N.W. Bass (Bredehoeft, 2005), "A geologic report is always a progress report", which means that with new data, the conceptual model has to be revised and/or the numerical model recalibrated.

ACKNOWLEDGEMENTS

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Novel Physico-Chemical Techniques of Soil and Water Protection and Remediation

4.5. Metal binding by humic substances and dissolved organic matter derived from compost
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CURRENT AND FUTURE IN SITU TREATMENT TECHNIQUES FOR THE REMEDIATION OF HAZARDOUS SUBSTANCES IN SOIL, SEDIMENTS, AND GROUNDWATER

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Abstract: The U.S. Environmental Protection Agency (EPA) Office of Research and Development (ORD) is the scientific research arm of EPA. ORD conducts research on ways to prevent pollution, protect human health, and reduce risk. Much of the research related to demonstration and evaluation of innovative cleanup technologies is conducted in ORD's National Risk Management Research Laboratory. One of the mechanisms for the evaluation of innovative field -scale technologies for hazardous waste remediation is the Superfund Innovative Technology Evaluation (SITE) Program. The SITE Program is currently investigating approaches for the *in situ* treatment of contaminated sites. The research includes innovative technologies for clean up of dense non-aqueous liquids (DNAPLs) in soils, groundwater, and fractured rock media; innovative approaches for contaminated sediment remediation; and innovative mine waste treatment technologies. Future research includes the evaluation of remediation of DNAPL source zones; treatment caps that simultaneously contain and remediate contaminated sediments; and investigation of ecological tools that serve a dual purpose of assessing the condition of sediment -contaminated water bodies and gauging the efficacy of mitigation efforts. These efforts are leading to a better understanding of soil, sediment and groundwater systems that may respond innovative treatment technologies, and more accurate information regarding the performance and cost of these innovative approaches.

Key words: Innovative cleanup technologies; SITE Program; *in situ* treatment; clean up of DNAPLs; sediment remediation; mine waste treatment; future research; performance and costs of clean up

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1. INTRODUCTION

Following increased public awareness of in the 1970’s of the national problem of abandoned wastes, the United States Congress enacted the Comprehensive Environmental Response, Compensation and Liability act of 1980 (CERCLA) and the Superfund Amendments Reauthorization act (SARA) in 1986. These laws and other related laws established a federal program for preventing, mitigating, and responding to releases of hazardous substances that threaten human health and the environment.

Since the 1980’s hazardous waste treatment has progressed from incineration for contaminated soils and pumping followed by above-ground treatment for groundwater. By 1985 it was clear that existing strategies aimed at cleaning up contaminated sites were not working as effectively as required. Each of those alternatives had problems inherent to the technology: incineration produces air emissions and ash that need to be controlled and handled, and the pump-and treat-technologies were projected to last for 20 years or more, making the cost prohibitive at many sites.

The United States Environmental Protection Agency (EPA) functions as both a scientific and regulatory agency of the United States. Research conducted under the EPA's Office of Research and Development (ORD) provides the basis for the formulation of environmental policies and programs. ORD's National Risk Management Research Laboratory (NRMRL) plays a vital role in the scientific research mission at EPA.

The mission of the National Risk Management Research Laboratory is to develop ways to prevent pollution of air, land, and water, and to restore ecosystems. The NRMRL research is directed toward a wide range of environmental challenges in seven research areas:

- Drinking Water Protection
- Air Pollution Control
- Contaminated Media Remediation
- Pollution prevention
- Watershed Management and Protection
- Environmental Technology Verification and Evaluation
- Technology Transfer and Technical Support

2. TECHNOLOGY EVALUATIONS AND VERIFICATIONS

The Superfund Innovative Technology Evaluation (SITE) Program was established by the Superfund Amendments and Reauthorization Act of 1986 to address the need for innovative technologies for the cleanup of Superfund and other hazardous sites. The initial priority of the SITE Program is research, development, and evaluation of innovative remediation technologies that are faster, safer, and more cost effective. The second priority is to develop more accurate techniques for assessing sites to determine the nature and extent of environmental contamination.

The SITE Demonstration Program conducts field demonstrations and high-quality performance verifications of viable remediation technologies at sites that pose high risks to human health and/or the environment. The program solicits applications annually from those responsible for cleanup operations at hazardous waste sites and develops a list of media and contaminant emphasis areas where innovative technology research and development is most needed. The contaminant focus areas are listed in Table 1.

Table 1. Contaminant emphasis areas for 2000-2006.

Surface Water/Groundwater	Soils/Sediments
DNAPL/chlorinated solvents	Pesticides
(PCBs)	PCBs
Arsenic, mercury or other heavy metals	Arsenic, mercury or other heavy metals
	(PAHs)

The Environmental Technology Verification (ETV) program provides credible performance data for commercial-ready environmental technologies to speed their implementation for the benefit of vendors, purchasers, permitters, and the public.

SITE Innovative technology evaluations are funded by financial and in-kind contributions from the project stakeholders. The SITE Program is responsible for development of a demonstration plan, sampling, sample analysis, and report writing. Technology vendors are responsible for operation and maintenance of the technology. Site/problem owners are financially responsible for site infrastructure and waste disposal. The ETV Program is carried out through agreements with private testing and evaluation organizations. ETV conducts verification activities for a broad range of technology categories, including monitoring and treatment technologies relevant for homeland security. The resulting data and reports from these programs are intended for use by decision-makers in selecting remediation options and for increasing credibility in innovative applications.

3. TREATMENT TECHNIQUES CURRENTLY UNDER EVALUATION

NRMRL Research Programs conduct evaluations and verifications of innovative hazardous waste treatment technologies in collaboration with industry, academia, and other federal agencies. The Programs identify the most pressing problems at hazardous waste site and seek technologies to address those problems. A number of innovative treatment technologies have shown promise in pilot and field applications. These are currently being evaluated to assess their performance, cost, applicability, ability to implement, state and community acceptance, and compliance with regulatory requirements. Additionally, as these technologies are experience continued development, evaluations are conducted to assess process optimization, and to determine long term effectiveness and permanence.

4. *IN SITU* TREATMENT TECHNIQUES FOR SEDIMENTS

Contaminated sediment has significantly impaired the navigational and recreational uses of rivers and harbors in the U.S. (NRC, 1997 and 2001) and is a contributing factor in many of the 2,800 fish consumption advisories nationwide (U.S. EPA, 2003). As of 2001, EPA had decided to take action to clean up sediment at approximately 140 sites under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and additional sites under the Resource Conservation and Recovery Act [(RCRA), U.S. EPA, 2001.

In situ capping refers to placement of a subaqueous covering or cap of clean material over contaminated sediment that remains in place. Caps are generally constructed of clean sediment, sand, or gravel, but can also include geotextiles, liners, or the addition of material, such as organic carbon, to attenuate the flux of contaminants. *In situ* treatment caps perform remediation of contaminated media due to the nature of the interaction of the capping material with the subaqueous sediments. These chemical interactions either destroy the contamination or sequester contaminants through a combination of adsorption, absorption, ion exchange, and precipitation.

The Site Program is investigating various subaqueous capping techniques at sites in Connecticut and Washington D.C. In addition, the program is also evaluating the use of electrochemical oxidation techniques, sediment washing, and a thermal sediment reuse technology. Other research within the

National Risk Management Laboratory investigates issues related to dredging of contaminated sediments and monitored natural attenuation. In addition to numerous other contaminated sites, this research will address the 215 National Priorities List (NPL) sites that contain polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and pesticides in sediments, 223 NPL sites that contain mercury and other hazardous metals in sediments, and the 31 Great Lakes Areas of Concern (AOCs) with sediment contamination.

5. ADVANCED PHYSICAL/CHEMICAL TREATMENT

Many new technologies are under development in the area of physical and/or chemical treatment of contaminated matrices. Many of these technologies remain unproven or are at the developmental phases. Using these technologies can expand *in situ* cleanup opportunities to medium and low permeability soils, semi-volatile organic compounds (SVOCs), volatile organic compounds (VOCs). Additionally, these technologies have applicability to remediation of metals and areas where excavation costs are prohibitive or excavation is infeasible. These technologies include:

- *In situ* chemical oxidation involves the use of various oxidants and delivery techniques in various combinations to destroy heavy organic compounds. The method involves thoroughly permeating the contaminated zone with sufficient quantities of chemical oxidants so that the chemical can contact and fully react with contaminants. Oxidants used in the systems include potassium and sodium permanganate hydrogen peroxide, and ozone.
- *In situ* extraction techniques are used to mobilize heavy organic-based contaminants such as DNAPLS. These techniques are designed to effect rapid mass transfer from the immobile contaminant into a mobile phase. The subsurface may be heated by either resistive heating techniques of steam. Another technique is the subsurface injection of cosolvents or surfactants in order to lower the interfacial tension between the contaminant and the soil while increasing contaminant solubility in water.
- *In situ* delivery systems such as directional drilling to place wells under active surface structures or in horizontal positions for increased injection or sparging efficiency are important in areas where buildings or other structures cannot be removed.

- Permeable reactive barriers (PRBs) are an *in situ* treatment technique where contaminated groundwater flows through a reactive zone. The contaminants are either mobilized or chemically transformed to less toxic compounds. The PRB serves as a barrier to the contaminants, but not to the groundwater flow.

The SITE Program is currently evaluating the efficacy and cost of *in situ* oxidation and dehalogenation techniques at demonstrations in New Hampshire, Connecticut, and Texas. These projects address contamination in soils and groundwater that is prevalent at 802 sites on the final NPL that contain hazardous volatile organic compounds and dense non-aqueous phase liquid contamination in plumes and source zones.

6. BIOREMEDIATION

Bioremediation involves chemical transformations mediated by microorganisms that degrade organic contaminants in either excavated or *in situ* soil, sludge, and solids. The microorganisms break down contaminants by using them as a food source or cometabolizing them with a food source. In some instances, bioremediation is used with other technologies to accomplish a greater total removal efficiency of organic contaminants. Bioremediation projects address issues related to cleanup of contamination in soils and groundwater that is present at 813 sites on the final NPL that contain hazardous organic compounds, as well as other state and federal sites with organics contamination. Additionally, bioremediation projects are conducted under NRMRL’s Oil Spill Bioremediation Program research area to evaluate its effectiveness for cleaning up and restoring inland wetlands, coastal salt marshes, and sediments contaminated with crude or refined oil.

7. PHYTOREMEDIATION

Phytotechnology is a set of technologies using plants (roots, shoots, tissues, and leaves) to remove, transfer, stabilize, or destroy contaminants in soil sediments and groundwater. Phytoremediation applies to all biological, chemical, and physical processes that are influenced by plants that aid the cleanup of contaminated substances. The specific phytotechnology is chosen based on the type of contaminated media that are affected and the remediation goals. Remediation goals include areas such as containment, stabilization, sequestration, assimilation, reduction, detoxification, degradation, mobilization, and /or mineralization.

Plants naturally remove man-made contaminants through several mechanisms. Some plants degrade organic pollutants directly or indirectly by supporting microbial communities. Other plants take up organic contaminants from soil or water and concentrate them in the plant tissue where the contaminant can be removed and disposed of separately, leaving the soil clean. U.S. EPA National Risk Management Laboratory tested and evaluated the performance of the technology at a number of field sites. The SITE Program recently evaluated the technology for remediation of groundwater at Air Force Plant 4, Carswell, Texas. The study was undertaken to determine the potential for a planted system to hydraulically control the migration of contaminated groundwater, as well as biologically enhance the subsurface environment to optimize *in situ* reductive dechlorination of chlorinated ethenes present (trichloroethene and cis-1,2-dichloroethene) in the shallow aquifer system (EPA, 2002b).

8. FUTURE CHALLENGES

One of the critical needs for remediation technology is for methods to accelerate aquifer cleanup. Groundwater contamination may consist of multi phase plumes, light non-aqueous phase liquids (LNAPLs), dense non-aqueous phase liquids (DNAPLs), which can potentially move in different directions. These contaminants can be difficult to locate in the subsurface, and small amounts can contaminate large volumes of an aquifer. Many unanswered questions remain regarding the efficacy of contaminant source zone treatment in reducing the time required for remediation of aquifers, particularly the short and long term impacts on the rate of contaminant mass discharge or flux emanating from the source zone.

The persistence of PCBs in soils and sediments underscores the need for ecological indicators to assess the success of risk management strategies. Biological indicators have historically been used to assess the ecosystem condition and the impact or adverse effects of a contaminant. However, indicators are needed in the remediation and risk management of contaminated sediments as a measure of progress and success of a risk management strategy. Indicators and monitoring are useful for source identification and control, evaluating fate and transport, and assessing the performance of monitored natural recovery.

The SITE Program continues to focus on the need for technologies capable of *in situ* remediation of DNAPL in difficult geological formations. The program will continue to evaluate *in situ* thermal and chemical oxidation type technologies under a broad array of geological conditions. In addition, effective remediation technologies for metals in soils, treatment of recalcitrant compounds, and the general need for *in situ* treatment remain high priorities.

9. CONCLUSIONS

NRMRL continues to address existing environmental problems while looking ahead to potential future challenges. As the human population continues to grow and development continually increases, the task of protecting the environment becomes even more challenging and complex. The focus of environmental protection has shifted from regulating single chemicals to developing a multimedia approach to understand the synergistic effects of hazardous compounds distributed throughout the environment. The Laboratory continues to seek innovative environmental solutions and to conduct research designed to address gaps in scientific knowledge.

NRMRL research programs will look to address future challenges while continuing to conduct research, development, testing, evaluation, and comparison of remediation technologies, thus reducing costs and accelerating technology development and use. The Laboratory's strong relationships with national and international research and development communities will continue to play an important role in responding to emerging environmental issues.

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LONG-TERM PERFORMANCE OF PERMEABLE REACTIVE BARRIERS: LESSONS LEARNED ON DESIGN, CONTAMINANT TREATMENT, LONGEVITY, PERFORMANCE MONITORING AND COST – AN OVERVIEW

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Abstract: An overview of permeable reactive barrier (PRB) performance for field sites in the U.S. was evaluated over the last 10 years by the U.S. Environmental Protection Agency's Office of Research and Development (EPA-ORD) in collaboration with other U.S. federal agencies, consulting companies and academic institutions under activities sponsored by the EPA's Remedial Technology Development Forum (RTDF). The RTDF is a public-private partnership which undertakes research, development, demonstration, and evaluation efforts focused on finding innovative solutions to high priority environmental problems. The focus has been on evaluating the effectiveness of these systems for plume capture and contaminant degradation/immobilization, microbiological and geochemical impacts on reactivity and longevity, and cost. Challenges for future research, development and application of PRBs are also presented.

Key words: permeable reactive barrier; zero-valent iron; ground water; remedial technology arsenic; long-term performance assessment

1. INTRODUCTION

Permeable reactive barriers (PRBs) have been the most successful, innovative in situ remedial groundwater technology that has been developed

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during the past two decades. During the past several years PRB technology has been investigated by the USEPA for their long-term performance capability, their ability to treat a wide variety of contaminants, innovative approaches with respect to emplacement including applications of this technology at depths in excess of 100 m, the adaptation of the technology for source zone remediation and studies of the microbial ecology of these systems and their impacts on downgradient water quality. These studies have contributed to the development of this technology and assisted with the advancement of the technology throughout the world. To date, more than 100 PRBs have been installed worldwide. Most of these installations have been successful at accomplishing remedial goals. A few sites have encountered problems but these problems can typically be attributed to design shortcomings due to poor site characterization, poor design selection or used at sites where the technology was not an appropriate choice.

2. ADVANCEMENTS IN DESIGN AND CONSTRUCTION

Permeable reactive barriers have been an attractive choice for many waste sites as a containment measure to stop the advance of ground-water plumes, while at the same time treating the contaminants in place using natural processes such as reductive chlorination for chlorinated solvent compounds or adsorption or precipitation reactions for metals. The advantages of this approach include treatment of contaminants in the subsurface, complete plume capture, a passive (low energy) treatment approach that has considerably lower operation and maintenance costs and lower long-term performance monitoring costs (Powell et al., 2002). The latter cost savings accrue due to the focused nature of the monitoring network designs and the ability to reduce monitoring frequency over time.

Construction methods for PRBs have expanded from the original funnel-and-gate type design using typical excavation equipment to include other methods such as continuous deep trenching machines, deep soil mixing, vertical hydraulic fracturing, jetting, vibrated beam, and bioslurry methods. Today, the most common methods of installing PRBs are excavation using biodegradable slurry for support of the excavation, continuous trenching machines, and vertical hydraulic fracturing.

The lessons learned from previous PRB applications have implications for PRB construction. Certain construction methods, when used at sites where the PRB has to be installed through low-permeability layers, have the potential for smearing. For example, if sheet piling has to be driven through low-permeability layers during construction of a PRB, there is potential for smearing and potential consequent flow impedance. Trenching (supported by biodegradable slurry) may be a better method at such sites. In addition, sharp permeability contrasts should be avoided. Generally, the simplest PRB configuration is usually the best, namely, a single trench (or space) filled with a uniformly sized reactive media.

Installation of a granular iron PRB using biopolymer or biodegradable slurry is similar to constructing a conventional impermeable slurry wall. As the trench is excavated, biopolymer slurry is added as liquid shoring to provide stability for the trench walls. The biopolymer slurry used is typically guar gum based. Excavation continues through the biopolymer without the need for dewatering. The reactive media is placed through the slurry by tremie. Any residual slurry in the PRB is broken down by circulation of an enzymatic breaker fluid and through natural biological degradation allowing groundwater to flow through the PRB. Depths of up to 27 m and a thickness of 0.6 m or greater can be achieved using this method.

Vertical hydrofracturing enables the placement of PRBs deeper than possible by conventional construction methods of open trenching or biopolymer supported trenching. Continuous PRB treatment walls deeper than 100 m 22 cm thick can be injected into the subsurface using vertical hydrofracturing. This installation method is minimally invasive (i.e., no trenching), requiring only the drilling of 15 cm boreholes approximately every 4-5 m on the planned placement line of the PRB. Due to minimal site disruption and depth capabilities, vertical hydrofracturing is ideally suited to treat deep contaminant plumes and/or emplace PRBs in urban settings where surface structures and underground utilities may make surface trenching impractical. The vertical interval for fracturing and injection is isolated in the borehole by packers and the PRB is built from the bottom up. Iron filings of medium sand size are mixed with hydroxypropylguar (HPG) biodegradable gel and immediately before injection, a special enzyme and cross-linker are mixed with the HPG gel and iron filings to form a highly viscous gel. The gel and iron filings are then injected under low pressure creating a fracture pathway for the gel and iron filings to follow. The enzyme breaks down the gel within a few hours, reducing it to water and harmless sugars, leaving a permeable wall of iron filings. Vertical hydrofracturing has been used up to 38 m below ground surface (bgs) and to 370 m in length.

3. SITE CHARACTERIZATION AND LONG-TERM PERFORMANCE

More so than conventional pump-and-treat type remedies, PRBs require much better understanding of site characteristics. PRBs are a passive technology. By their very nature, PRBs are expected to take advantage of natural groundwater flow conditions to bring the contamination to the reactive medium. Once installed, this passive system is comparatively more difficult to modify or adjust than a pump-and-treat system. Therefore, there is a greater need in ensuring a successful PRB design than there is in a pump-and-treat system. Lessons learned from previous applications need to be appropriately incorporated in future design of PRBs and the associated monitoring systems. The two site factors most important to consider are hydrology and geochemistry.

3.1 Site Hydrology

Understanding the groundwater flow regime is the key to the physical design of a PRB system. Most "well-characterized sites" have good water level maps on a moderately large scale. However, to ensure that the PRB is oriented as perpendicular to the flow so that it captures the maximum volume of groundwater, data on a more localized scale (few to tens of meters) are required. Because most PRBs are built to a thickness of 2 m or less, groundwater flow information at this scale is required. At such a small scale, local aquifer heterogeneities play a much larger role in determining flow. Depending on the presence of tidal and/or seasonal influences at a site, groundwater flow magnitude and direction can change substantially. Generally, it is advisable to obtain at least four quarters of water level data for adequate design of the PRB, to account for seasonal variability. Where tidal influences are possible, monitoring frequency needs to be adjusted accordingly.

At most sites, aquifer properties in the region of interest often tend to vary by one or two orders of magnitude. At many sites, average values of these aquifer properties have been used to determine an average groundwater velocity that is then used in the PRB design. Generally, a better approach is to collect highly resolved vertical flow estimates along the projected barrier path emplacement and conduct groundwater and solute transport modeling and simulate a whole range of flow conditions to determine flow and best design the orientation and dimensions of the PRB.

3.2 Site Geochemistry

Geochemistry is important from the perspective that other groundwater constituents (constituents other than the target contaminants) may interfere with a PRB's ability to perform its two main functions:

- Capture groundwater from the targeted portion of the aquifer and provide it with sufficient residence time in the reactive medium
- React with the target contaminants and reduce their concentrations to target cleanup levels.

Dissolved inorganic constituents, such as oxygen, calcium, magnesium, carbonates, and sulfates, in the groundwater have a tendency to react with zero-valent iron in PRBs. Over long periods of time, water itself reacts with iron, leading to a number of transformations in the iron-water system. These reactions have the potential to limit the hydraulic and reactive performance of the PRB. The first evidence that iron was impacting more than just the target contaminants came from groundwater monitoring of the influent and effluent water from iron PRBs. A comparison of groundwater chemistry between upgradient and downgradient wells indicates that the iron media at Elizabeth City, North Carolina, United States, is a long-term sink for C, S, Ca, Si, Mg, and N. Solid phase characterization studies indicate average rates of inorganic carbon and sulfur accumulation of ~0.1 and ~0.05 kg/m^2y at Elizabeth City (Wilkin and Puls, 2003) where upgradient waters contain up to 400 mg/L total dissolved solids. Carbon accumulation rates, based upon the solid-phase characterization studies, are in good agreement with estimates made through reactive transport modeling efforts (Blowes and Mayer, 1999).

A number of field studies have now shown that the strongly-reducing (low-ORP) and high-pH conditions, created when zero-valent iron contacts water, causes several inorganic constituents to transfer from the solution phase onto the iron (Puls et al., 1999; Wilkin and Puls, 2004). This can lead to formation of molecular or particulate films on the iron surfaces. Calcium carbonates, iron carbonates, and iron hydroxides are among the insoluble mineral species that form and deposit on the iron. Not only is iron mass consumed in these reactions, but the formation of potentially passivating films on the iron surfaces can reduce the reactivity of the remaining iron mass. The implication of this observation was that the reactivity of the PRB could potentially decline, even while considerable iron mass still remained in the PRB. Subsequent studies have shown that some of the corrosion films that form on the iron surface are themselves reactive and degrade certain contaminants (Su and Puls, 2004). However, in the longer term, reactivity is expected to decline.

Typically, iron cores collected after a few years of field operation of the PRBS have been analyzed by specialized methods, such as x-ray diffraction to determine crystalline mineral phases and scanning electron microscopy to identify specific compounds on the iron grains. At Elizabeth City, and several other sites, where detailed examination of the used iron medium has been conducted, the types of precipitates identified have been relatively similar and have consisted primarily of calcium carbonates, iron hydroxy carbonate, carbonate green rust, hydrous ferric hydroxide, ferric oxyhydroxide, and iron monosulfides (mackinawite and greigite) (Wilkin and Puls, 2003). Microscopy observations indicate that mineral accumulation mainly occurs on the surfaces of the iron particles collected near the upgradient aquifer/iron interface where steep gradients in pH and redox potential promote mineral nucleation and growth. Geochemical conditions that may pose serious constraints on PRB longevity include: high sulfate, high carbonate, and high nitrate concentrations in ground waters. The physical presence of precipitating mineral species in the PRB pore space will eventually affect the hydrology of the PRB. Clogging of the pores in the reactive medium has the potential to cause reduction in residence time of the groundwater and target contaminants and may lead to mounding of groundwater in the upgradient aquifer with potential bypass flow around, over, or under the PRB.

3.3 Site Microbiology

Microbiological impacts are also important to understand in order to better predict how long PRB systems will remain effective. The presence of a large reservoir of iron coupled with abundant substrate availability (i.e., hydrogen) supports the metabolic activity of iron-reducing, sulfate-reducing, and/or methanogenic bacteria. About 35 core samples collected from the Elizabeth City PRB were analyzed for content and distribution of phospholipid fatty acids (PLFA). These organic compounds can be used as lipid biomarkers to provide a quantitative means to evaluate viable microbial biomass, community composition, and nutritional status. Biomass concentrations after 4 years at Elizabeth City ranged between about 5 and 875 pmoles of PLFA per gram of dried iron, or between 1.02×10^5 and 1.78×10^7 cells per gram of iron matrix. The highest concentrations of microbial biomass were found at the upgradient aquifer/iron interface. Analysis of PLFA structural groups suggests the dominance of anaerobic, sulfate-reducing and metal-reducing bacteria. Low concentrations of microbial biomass in mid-barrier and downgradient samples suggest that the environment at these locations is more challenging to bacterial growth and survival.

Core samples collected from the Denver Federal Center PRB were also analyzed for content and distribution of phospholipid fatty acids (PLFA). The highest accumulations of microbial biomass were found at the DFC Gate 2 near the upgradient iron/aquifer interface, where concentrations were as high as 4,100 pmoles of PLFA per gram of dried iron (8.36×10^7 cells/gm). The analysis again suggests the dominance of anaerobic, sulfate-reducing and metal-reducing bacteria. The USEPA has ongoing studies at a number of PRB sites to further evaluate the effect of PRBs on the microbial ecology of these systems and the effect of biological activity on PRB longevity.

4. DOWNGRADIENT AQUIFER ‘CLEAN UP’

In many cases, PRBs are installed within a plume rather on the distal end of the plume, often prevent further migration off site or to protect a vulnerable receptor. In these cases it is important to understand that aquifer contamination downgradient of the barrier wall will persist for an extended time frame due to slow desorption of the contaminants from the aquifer matrix. The extent of time for the downgradient aquifer to “clean up” will be a function of the physical-chemical characteristics of the contaminant(s), the nature of the aquifer sediments, the ground water flow rate, and the effectiveness of the PRB itself. At the Elizabeth City, North Carolina site in the U.S. it took less than 2 years for the downgradient chromate concentrations to decrease below remedial goals but it took the TCE almost 7 years to attain remedial clean up goals.

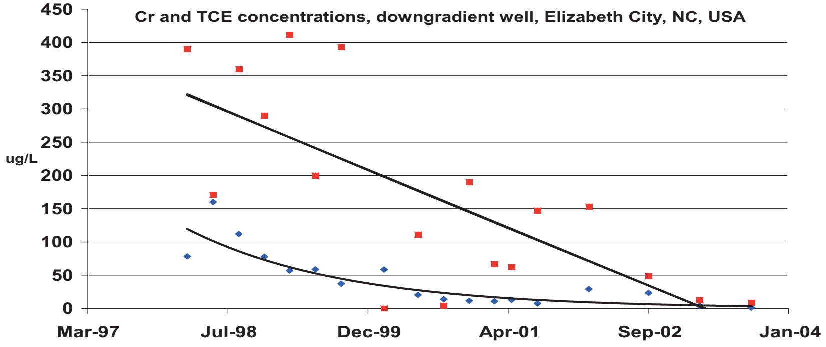

Figure 1. Cr (diamonds) and TCE (squares) concentrations over time are shown for a downgradient well from the permeable reactive barrier at the Elizabeth City, NC, USA site.

5. CURRENT AND FUTURE USEPA PRB RESEARCH

Current USEPA research is focused on the following areas:

- PRB long-term performance
- Monitoring methodologies to further decrease operation and maintenance costs
- Microbiological effects and impacts on PRB performance
- Combining PRBs with other remedial technologies
- Dense non-aqueous phase liquid (DNAPL) source zone remediation
- Applications for arsenic, other inorganic contaminants, and mine wastes
- Protection of coastal ecosystems

Pilot field tests are ongoing for arsenic treatment, lead treatment, nitrate treatment (confined animal feeding operations), and DNAPL source zone treatment. The latter project was recently initiated and is using nano-scale iron to treat a chlorinated solvent site in South Carolina, U.S.

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USING ABUNDANT WASTE AND NATURAL MATERIALS FOR SOIL AND GROUNDWATER PROTECTION AGAINST CONTAMINATION WITH HEAVY METALS

Prospects and Areas of Application

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Abstract: Sewage sludge (biosolids) application on land as a fertilizer and soil improver, and high-volume sulfidic mining waste disposal and use as a common fill are anthropogenic activities that increasingly contribute to heavy metal enrichment of soil and ground water. (i) Sewage sludge (SS) use in agriculture appears to be the most attractive and cost effective, but at the same time a controversial disposal outlet due to concentration of all kinds of anthropogenic pollutants, of these the heavy metal content has been of the major concern for many years. While large industries in principle achieved required source control of heavy metals in this field, small artisan enterprises (e.g. metal electroplating, vehicle repair workshops, etc), still contribute significantly to the heavy metal loads in SS. Experiments on metal sorption onto natural organic matter (NOM) such as high-moor peats and SS in batch and flow-through systems from different, low-pH, polymetallic electroplating waste solutions (EPW) revealed high viability of this process. Use of small amounts of organogenic materials, preferably waste such as SS as a sorbent to be incinerated after use, could greatly and at almost no costs improve quality of bulk SS to enable its environmentally sustainable use in agriculture. (ii) Emission of heavy metals and sulfate loads from other objects of concern (sulfidic mining waste dumps and civil engineering constructions) in all stages of their construction and in the post-closure period can be successfully attenuated with use of fly ash (FA) from coal-fired power plants that is another abundant bulk waste, and the aforementioned organogenic materials. Excellent insulating properties of properly placed FA: water dense mixtures with respect to air penetration (1-2 orders of magnitude higher than that of natural cohesive soils) can be utilized in protective layers in order to prevent access of air as a major reagent in

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sulfide oxidation resulting in heavy metals release and migration with leachate. These means can efficiently protect ground waters against long-term contamination by leachate from sulfidic waste, usually lasting for decades. The presented applications of waste materials comply with the nowadays policies and strategies focused on waste reuse and natural resources protection, with the sustainable development as a major target.

Key words: heavy metals; waste materials; natural organic matter (NOM); sewage sludge; sorbents; sulfidic mining waste; fly ash; insulating/reactive protective layers

1. INTRODUCTION

Only over the last three decades of the last Millennium mankind became increasingly aware of the limited sustainability of ecosystems being under continuously growing human impact (UNDP et al., 2000). One of the threats to sustainable development is long-term non-point soil and ground water contamination with Potentially Toxic Elements (PTE), i.e. heavy metals, resulting from the growing generation of wastes and specific waste management practice. Anthropogenic activities that increasingly contribute to heavy metals enrichment of soil and groundwater are in particular: (i) sewage sludge (biosolids) application on land as a fertilizer and soil improver; (ii) high-volume sulfidic mining waste disposal and use as a common fill.

An optimum solution of these problems is a strict source control of pollutants entering the environment from all anthropogenic sources.

2. USE OF SEWAGE SLUDGE IN AGRICULTURE

2.1 Current Status of Sewage Sludge Generation and Use

In the last three decades, the number of households connected to sewers and the treatment level in the OECD countries are constantly growing. Particularly high increase of quantitative and qualitative rates of sewage treatment occurred in the EU Member States due to progressive implementation of the Urban Waste Water Treatment Directive 91/271/EEC (1991). In 2002, in 16 of 29 OECD countries $\geq 70\%$ population was connected to waste water treatment plants (WWTP), of this in 12 countries of OECD Europe that are all the EU Members or Associate States. In 6 of

EU-15 Member States from 77% (Austria) to 88% (Germany) of waste comprised tertiary treatment with nutrient removal (OECD, 2005). This resulted in an increase of sewage sludge (SS) generation, from about 5.5Mt, d.m. (dry matter) in 1992 (EUROPA-Environment, 2002) to 8.8 Mt in the EU-15 and 9.9 Mt, d.m. in OECD Europa in 2000-2002. Currently, the highest documented amounts of SS (d.m.) are generated in the USA, Germany, Japan and Korea. Of the OECD countries, the longest tradition of use of SS in agriculture is in the USA (OECD, 2005).

The EU Sewage Sludge Directive 86/278/EEC (EEC, 1986) encourages the use of treated SS in agriculture in a regulated environmentally safe way and sets limit values for concentrations of heavy metals in SS and in sludge-treated soils. Currently, more stringent regulations have been under development (EC DG ENV, 2000). There are evident merits, but also drawbacks of SS use in agriculture that cause reluctance to approach this way of SS reuse in many countries. On one hand, SS is a source of valuable organic matter and nutrients, and in the microelement-deficient areas also of essential metals. On the other hand, due to their chemical properties, SS is a sink of many anthropogenic pollutants. Of these, heavy metals are of major concern due to persistence, high accumulation potential and their degree of toxicity to plants, animals and humans.

The agricultural reuse of SS in OECD and the EU-15 countries is diverse and ranges widely. For the EU-15 it is estimated to account for about 40% of the total generation (EUROPA-Environment, 2002). Of 21 OECD countries, 11 (among them Luxembourg, UK, Denmark, Norway and USA) use SS in agriculture in high amounts, from 39 to 87%, while in the other 10, among them Germany, Sweden, Finland, and Japan, incineration or landfilling prevail over the agricultural use due to the focus on soil protection (Figure 1).

New EU member states that joined the EU in 2004 are not uniform with respect to the share of SS being used in agriculture. The percentile range of this way of SS utilization is very broad, from the highest (87% in Slovakia and 77% in Czech Republic) to the lowest level (12% in Poland) (OECD, 2005).

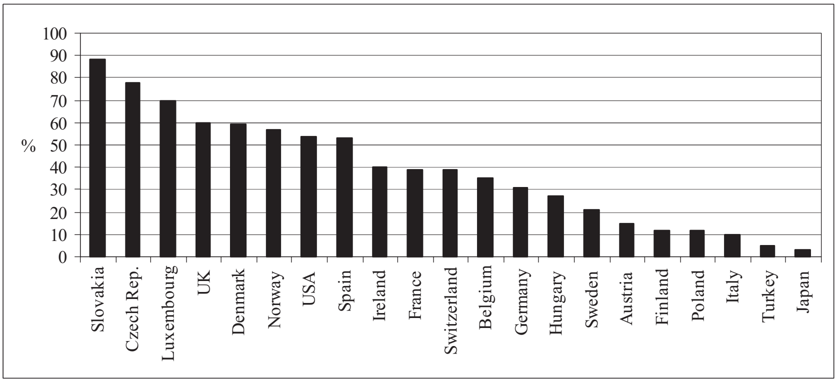

Figure 1. Share of sewage sludge use in agriculture in OECD and EU-15 Member States in 1993-2002 (after OECD, 2005).

2.2 Heavy Metal Source Control in Sewage Sludge

Heavy metals in sewage are mostly associated with suspended solids and enrich SS during treatment processes in WWTPs. An EC report (2001) identifies three major sources of heavy metals in urban wastewater and subsequently in SS (Table 1). All these sources contribute significantly to the total load of heavy metals, of this domestic/small artisan activities often dominate over large industrial/commercial sources (e.g. with respect to Zn, Pb, Cu, and Ni). Due to the efficient implementation mechanisms of the environment protection regulations, large industries in the EU in principle achieved required level of heavy metals source control. Its further improvement depends on the further development and compulsory implementation of BAT – Best Available Techniques. On the other hand, domestic sources, if not partly regulated centrally (e.g. by replacing pipes, taps etc.) are mostly non-controlled, while small artisan enterprises (e.g. metal electroplating, vehicle repair workshops) that significantly contribute to heavy metal loads in SS usually cannot sustain economically the sophisticated high-tech heavy metals removal applied in large industrial plants.

Therefore, development of easy-to-use, cost-effective, simple but highly efficient techniques to be commonly applied in small artisan enterprises might result in significant reduction of PTE in urban wastewater and consequently also in SS. The pollution potential of SS used as a soil improver might be thus successfully reduced. This was the rationale of the presented studies.

Table 1. Share of potentially toxic elements (heavy metals) from different sources entering urban wastewater treatment plants in the EU Member States (after EC, 2001).

Heavy	Load sources (% of the total input)*			
metals	Domestic/artisan	Commercial/Industrial	Urban run-off	Not identified
Cd	20–**40**	29–**60**	3–**41**	16
Cr	2–20	**35–60**	2–22	**61**
Cu	**30–75**	3–21	4– 6	29
Hg	4	**58**	1	**37**
Ni	10–**50**	27–34	9–16	**47**
Pb	**30–80**	2–24	29–**33**	**43**
Zn	**28–50**	5–35	10–16	**57**

* Major source percentiles are **bold**

3. HEAVY METALS SORPTION ONTO NATURAL ORGANIC MATTER (NOM) AND STABILIZED SEWAGE SLUDGE (SS)

3.1 Rationale

The idea of proposed technical solutions for small sources originated from the known capability of peat and other natural organic matter (NOM) to remove metal cations from wastewater (Couillard, 1994; Allen, 1996; Brown et al., 2000; Babel and Kurniawan, 2003). Peat was found to be an excellent sorbent of metals from small amounts of water and at low flow rates, i.e. for conditions appropriate for the aim under consideration. Since the use of peat is restricted by wetland protection requirements, this matter was used in these experiments as almost pure NOM to elucidate its sorption/remobilization properties under diverse conditions.

The major idea of the studies was application of SS as abundant, low-cost waste organogenic sorbent for source control of heavy metals by means of efficient treatment of wastewaters from artisan activities in simple installations, in order to reduce heavy metal enrichment in SS from WTTP to be used in agriculture as viable solutions for soil and ground water protection from non-point contamination. In short, using small amounts of SS for source control of heavy metals in wastewaters to reduce the loads and concentrations of these potentially toxic elements in bulk SS, thus improving its environmental safety.

Below, the results of these studies are briefly presented and summarized.

3.2 Materials and Methods

The experiments (batch and flow-through) comprised metal cation sorption onto NOM (peat) and municipal sewage sludge (SS) from real polymetallic electroplating waste solutions (EPW) of different pH and metal concentrations. The study was conducted with use of (i) 26 samples of high-moor peat of different botanical origin; (ii) an averaged and homogenized composite air-dried sample of sewage sludge (SS) from secondary treatment at WWTP and (iii)10 samples of real EPW from different enterprises, with diverse metal composition and contents, and pH range from 1.47 to 6.1.

Studies on peat were focused on behavior and mechanism of heavy metal sorption onto NOM, and for this reason were the most detailed with respect to the scope of organic matter characterization as a sorbent. It included: (i) major physico-chemical parameters assessed by the methods commonly used in NOM analysis; (ii) structural characteristics of NOM and identification of metal cations binding mechanisms with use of FTIR, NMR, potentiometric titration, HPCE, UV-VIS and fluorescence analysis. The aim of these studies was an elucidation of metal adsorptive properties of organogenic adsorbents for their optimum use. Results of the experiments of the more mechanistic nature that were reported and discussed elsewhere (Twardowska and Kyziol, 2003; Kyziol et al., 2006), were used for designing sorption studies of an applied character focused on the practical implementation of organogenic sorbents that are presented here. Selected parameters of NOM of particular importance for sorption properties are given in Table 2.

Table 2. Major physicochemical parameters of the studied NOM (high-moor peat W9b).

pH	DR (% vol.)	AC (% wt.)	Insoluble organics (g/kg)	Soluble organics HS (g/kg)	Humic acids HA (g/kg)	Fulvic acids FA (g/kg)	HA/FA
6.32	55	104.0	504.0	392.0	342.9	49.1	7.0
Cation exchange capacity							
CEC_0 cM/kg	CEC_t cM/kg	Base saturation (%)	Exchangeable ions, cM/kg, % CEC_t				
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	H^{+}
119.5	130.0	91.92	110.2 84.8%	8.50 6.54%	0.37 0.28%	<0.05	10.5 8.08%

DR – decomposition rate; AC – ash content; CEC_t – total cation exchange capacity; CEC_0 – base cation exchange capacity; HA – humic acids; HF – fulvic acids

Heavy metal contents in NOM were in low or trace amounts typical for pristine areas, while in sewage sludge (SS) these elements were highly enriched. Such metals as Fe and Mn occurred in both materials in comparable amounts (Table 3).

Table 3. Metal contents in studied oranogenic materials (mg/kg, d.m.).

Metal ions	Cd	Cu	Fe	Mg	Mn	Zn
22 peats	0.51−0.93	5.58−11.08	5250−14770	1120−1533	237−274	3.39−5.12
SS	10.70	306.5	17562	6330	200.7	1944

Here, sorption efficiency of metal cations onto NOM is exemplified in batch and flow-through laboratory experiments on a typical selected peat (W9c) and strongly acidic EPW(I) of pH 1.47. Batch sorption onto NOM was conducted at liquid/solid ratio L/S = 10:1. Flow-through experiments were performed under upward flow conditions through a fixed-bed column, at flow rate 0.1 mL/s and L/S = 2:1 dictated by the bed stability and porosity.

Sorption of heavy metals onto SS as a sorbent is illustrated by bench studies conducted in a simple batch reactor of 100 L volume with use of three EPW solutions (II-IV) of pH 3.50−4.75 and broad range of metal concentrations (Table 4), with a 2 h contact.

Table 4. pH and heavy metal concentrations in EPW(I-IV).

EPW	pH	Metal cations, mg/L							SO_4^{2-}.
		Cd^{2+}	Cu^{2+}	Cr^{3+}	Fe	Mn^{2+}	Ni^{2+}	Zn^{2+}	mg/L
I	1.47	171		235	15000	122		2810	7870
II	3.50	50	340	780			30	210	1220
III	3.70	53.4	172	193			14.9	163	
IV	4.75	4.75	19.0	10.4			3.39	121.6	

Binding strength of organogenic matter for metals was assessed by a 6-step sequential fractionation (Kersten and Förstner, 1986, 1988). Possible major mechanisms attributed to different binding strength onto NOM have been discussed elsewhere (Twardowska and Kyziol, 2003).

3.3 Results and Discussion

3.3.1 Metal Cations Sorption from EPW I onto NOM (Peat)

Literature sources consider the pH range 3.0 to 3.5 critical for most metal sorption onto peat (Allen, 1996; Brown et al., 2000). Studies on metal binding from synthetic monometallic Me-SO_4 solutions onto the same peat matter that was used in these experiments showed that the sorption ceased in a broader range of pH values strongly dependent on the metal ion: from pH 4.6 for Zn^{2+}, pH 4.5 for Cd^{2+}, through pH 3.5 for Cu^{2+} to pH 2.2 for Cr^{3+} (Twardowska and Kyziol, 2003).

Despite the considerably lower pH value of the polymetallic EPW I (1.47) than the lowest of the reported borderline values, all metals present in this solution were bound onto NOM, both in batch and flow-through systems. The sorption capacity of the studied NOM (peat) for a specific metal was determined by its input concentration in EPW and controlled by pH of the equilibrated solution.

Due to the specificity of a contact mode (10-fold excess of adsorbate over adsorbent and input metal loads limited to that in a single portion of adsorbate), equilibrium pH in the system reached a low value pH 1.67 close to that of EPW, thus the sequence of metal loads bound onto the NOM suspension appeared to correspond with their input amounts, while sorption efficiency of specific metals depended upon their critical pH value, at which the process terminated. For the studied solution (EPW I), the bound metal load sequence in batch process was: Fe>Zn>Cr>Cd>Mn, while with respect to the efficiency of binding onto peat (in % of input wt.) the metals can be ranked in an order: Cr>Fe>Cd>Mn>Zn (Table 5).

In the flow-through process, due to the longer period of non-equilibrium state and significantly higher input loads of metals entering the system before equilibration, both sorption capacity of the NOM (peat) and sorption efficiency for metals were higher than in the batch system, in particular those of Zn^{2+} and Mn^{2+} , i.e. metal cations particularly sensitive to pH (Table 5). While the metal sequence with respect to bound loads remained the same, though at much higher level, in particular with respect to Zn^{2+} and Cr^{3+}, the sorption efficiency of the peat matter for all metals but Cd^{2+} appeared to be almost even (80-84%), while sorption efficiency of Cd^{2+} was affected by Cr^{3+} competition.

Table 5. Summary of metals sorption (mg/kg, d.m) and its efficiency (% L_0)* onto the NOM (peat) from highly acidic EPW I (pH 1.47) under batch and flow-through contact mode.

Metal ions	Cd	Cr	Fe	Mn	Zn
		Batch			
Sorption S, mg/kg, d.m.	1050	2000	101000	355	3520
Sorption efficiency , % L_0*	61.3	85.3	67.5	29.1	12.5
Borderline pH value**	1.67	1.67	1.67	1.67	1.67
		Flow-through			
Sorption S, mg/kg, d.m.	2170	17400	126000	783	23000
Sorption efficiency , % L_0*	66.8	84.3	84.0	80.2	80.1
Borderline pH value**	1.93	1.42	4.00	1.68	4.29

* L_o – total input load of a metal; ** pH value at which sorption of a metal terminates

The difference of sorption pattern under batch and flow-through conditions is exemplified in Zn^{2+} binding onto peat W9b (Figure 2).

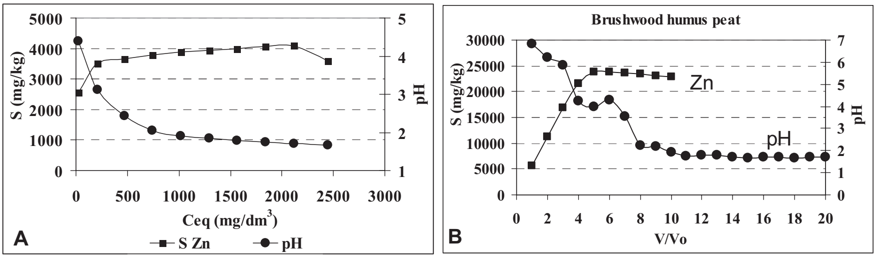

Figure 2. Sorption pattern of Zn under batch (A) and flow-through (B) conditions.

An important observation with respect to sorption behavior of the matter is the relative stability of metal loads bound under batch conditions at equilibrium pH value of solution 1.67 even with respect to Cd^{2+} and Zn^{2+} that are generally known to be easily remobilized.

Sequential fractionation of metals bound from the strongly acidic EPW I onto peat under batch contact mode showed their affinity mostly to two "pools": F5 – strong stable binding in metal-organic compounds and F2 – labile electrostatic and ion exchange binding, with significant part of the most labile retention in pore solution F0 (Figure 3). Fe was the only metal that enriched predominantly F4 "pool" attributed to amorphous Fe-oxides and chelate rings formation with HS functional groups.

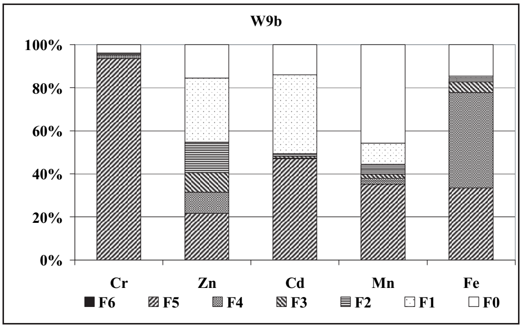

Figure 3. Sorbed metals fractionation with respect to binding strength onto NOM: F0 - the most labile, F1-very labile; F2 - mobile; F3+F4 - mobilizable, F5 - stable, strongly bound. Batch contact mode; Adsorbate: EPW I (pH 1.47).

Desorption of bound metals with 1% HCl (pH 1.38) confirmed the highest stability of sorbed chromium ions: Cr (87.3%) > Cd (78.3%) > Fe (42.0%) > Mn (36.1%) > Zn (26.5%). This sequence corresponds well with metal enrichment in the F5 "pool" – the highest for Cr^{3+} and the lowest for Zn^{2+}. The pattern of metal fractionation with respect to binding strength onto NOM from the strongly acidic EPW I under flow-through contact mode appeared to be similar despite of unequal sorption capacity for different metals.

3.3.2 Metal sorption from EPW II-IV onto stabilized sewage sludge

Metal sorption onto NOM (peats) has been thus proven to be an efficient process, in particular under a flow-through adsorbent – adsorbate contact mode that allowed also for a better utilization of NOM as an adsorbent. The requirements of wetland protection, though, imply the limitations of a wider use of natural materials and encourages seeking waste materials with a sufficiently high share of organic fraction as an adsorbent. Thus was an idea of using stabilized sewage sludge (SS) as an adsorbent for heavy metals to be used in small enterprises/workshops, despite already high enrichment of heavy metals in this matter (Table 3). The results of heavy metal removal from acidic EPW in a simple batch reactor, show high efficiency of SS as an adsorbent (Table 6).

Table 6. Efficiency of sewage sludge (SS) use as an adsorbent for heavy metal removal from acidic EPW II-IV in a simple batch reactor, at 2 h sorption cycle.

Heavy metal ions	Cd	Cu	Cr	Ni	Zn
Electroplating waste EPW II, pH 3.50					
Input concentration C_0, mg/L	50	340	780	30	210
Sorption S, mg/kg, d.m	790	606	1090	115	6750
Sorption efficiency , % L_0^*	96.6	97.3	98.2	87.5	92.8
Electoplating waste EPW III, pH 3.70					
Input concentration C_0, mg/L	53.4	172	193	14.9	163
Equilibrium conc. C_{eq}, mg/L	3.86	16.8	9.0	1.92	27.1
Sorption S, mg/kg, d.m.	495	155	1840	130	1360
Sorption efficiency , % L_0^*	92.8	90.2	95.3	87.1	83.4
Electroplationg waste EPW IV, pH 4.0					
Input concentration C_0, mg/L	4.75	19.0	10.4	3.39	122
Equilibrium conc. C_{eq}, mg/L	0.30	10.0	3.6	1.30	13.9
Sorption S, mg/kg, d.m.	44.5	90.0	68.0	20.9	1077
Sorption efficiency , % L_0^*	93.7	47.4	65.4	61.7	88.6

* L_o – total input load of a metal;

The results confirm high efficiency of metal sorption onto SS from acidic polymetallic EPW solutions in a batch process. The efficiency appeared to be higher for high input concentration of a metal in the EPW solution. Worse sorption efficiency at low input concentrations of metals in EPW results from a lower concentration gradient between adsorbent and adsorbate. Nevertheless, even at relatively low metal concentrations in EPW, the sorption efficiency was generally ≥ 50%; Cd removal exceeded 90% at all concentration ranges.

At a pH range 3.5-4.0 of EPW, which falls within the values that are considered optimum for the metal sorption process (Allen, 1996; Brown et al., 2000), binding of metals onto SS involved mainly "pools" F3+F4 of a moderate binding strength, associated with chelating complex formation with humic substances and sorption on amorphous Fe-oxides. The labile F1 "pool" also plays an important role, in particular in Ni and Zn sorption. Heavy metals that occurred originally in the SS showed similar fractionation (Figure 4).

To summarize, both NOM, but also organogenic waste, which is of particular importance, appeared to be efficient, easy-to-use and cost-effective sorbents for source control of heavy metals in effluents generated by small industries. An essential merit of waste organogenic sorbents is a possibility to substantially reduce hazardous waste volume by mineralization or incineration of spent sorbents (Twardowska et al., 2004a).

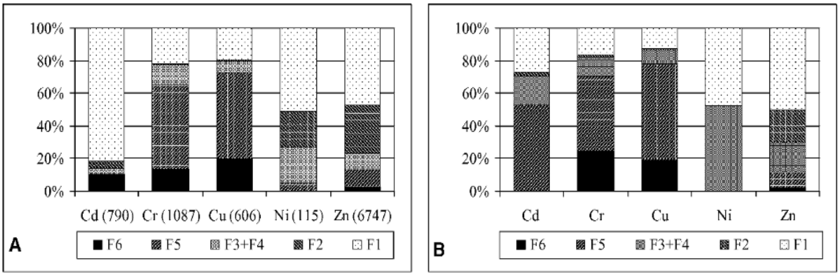

Figure 4. Metal fractionation in sewage sludge (SS) with respect to binding strength: (A) originally present in SS (pH 6.71); (B) bound from EPW II (pH 3.50). F0 - pore solution, the most labile; F1 - very labile; F2 - mobile; F3+F4 - mobilizable, F5 – stable, strongly bound.

4. USE OF WASTE MATERIALS FOR PREVENTION/ INTERCEPTION OF ACID ROCK DRAINAGE AND HEAVY METAL LEACHING FROM SULFIDIC WASTE DUMPS

4.1 Background and Rationale

Waste from the extractive industries constitute one of the largest waste stream in the world, one that is particularly problematic in mining areas. The

EU-15 that is a rather marginal producer of mineral commodities, generated annually over 400 Mt (million tons) of mining waste that accounted for about 29% of total waste stream (EEA, 2004). New members of the EU, among them Poland that is the biggest producer of hard coal in Europe and extracts also non-ferrous (copper) ores, and other countries that have developed extractive industries, might increase the amount of mining waste in the EU.

The world's minerals statistics and information can be found on the USGS web site (USGS, 2005). An overview of world mining output of principal non-metalliferous and metalliferrous minerals, of waste generated by extractive activities and related environmental problems are presented by Szczepańska and Twardowska (2004), while information on mining waste management practice is drawn up in official documents and guidelines (e.g. EC-JRC-EIPPC, 2004) and summarized in relevant literature sources (e.g. Hutchinson and Ellison, 1992; Twardowska et al., 2004b). The needs of control of the major environmental impacts associated with waste from extractive industries, namely water pollution issues and stability, gave rise to the development of a proposal for a Directive of the European Parliament and of the Council on the management of waste from the extractive industries (Commission of the EC, 2003).

The most serious environmental problem related to mining waste is acid rock drainage (ARD) generation resulting from sulfide oxidation in waste rock material exposed to air and water when extracted and disposed or used at the surface. The mechanism of iron sulfide oxidation is generally recognized as a multi-stage process that in its simplest form can be represented by equation (1):

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4\ SO_4^{2-} + 4H^+ \qquad (1)$$

The acid generation in sulfidic waste and the formation of sulfate-rich, highly mineralized leachate, with subsequent mobilization of metals and other dissolved contaminants is known as a long-term process lasting for decades that renders the receiving water bodies unfit for any use. Besides waste from extraction of metalliferrous minerals, also high-volume non-metalliferrous sulfidic waste, e.g. from coal mining, has the potential for long-term contamination of the aquatic environment by leachate of high total dissolved solids (TDS), mainly sulfates (Szczepańska and Twardowska, 2004).

Due to the scale and time of mining waste facility construction, and in particular because of specific characteristics of the waste material, general guidelines on waste landfilling are not applicable to sulfidic waste from extractive industries.

The idea of the method presented below is to use blanket-like preventive layers of waste material of low permeability to air evenly covering the top of each subsequent layer of extractive waste during a dump construction to prevent air penetration and thus to terminate the oxidation reaction (1).

4.2 Fly Ash Dense Mixtures with Water as a Sealing Material Against Air Penetration

Fly ash (FA) from coal-fired power plants applied as a dense mixture with water of the lowest quality, e.g. from the mine dewatering system, after solidification appeared o be a perfect insulation material against air penetration. The FA: water ratio used for a dense mixture preparation should assure its transport and spreading in a blanket-like insulation layer at the top of the subsequent mining waste layers to be protected against air penetration after finalizing the layer formation and leveling its surface.

Penetration resistance R of the solidified "pure" FA: water mixtures appeared to be about an order of magnitude higher than that of the natural cohesive soils used for sealing/insulation purposes (Figure 5). Admixture of

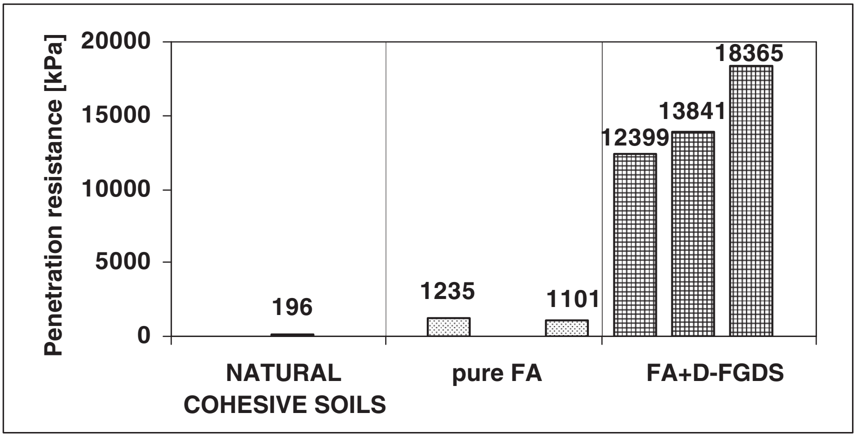

Figure 5. Penetration resistance (R) values of the solidified mixtures of FA with mine water (1:1 wt.) vs. that of natural cohesive soils (boulder clay).

products from dry flue gas desulfurization process in FA (FA+D-FGDS) causes significant enhancement of insulating properties of the solidified mixture and reduction of the solidification time that is of particular importance in areas with an unstable climate. As dry desulfurization process is not widely applied due to its low efficiency, an addition of lime, or preferably lime-containing waste to FA would give the same setting effect.

Being excellent insulating agents against air penetration, solidified FA: water dense mixtures are at the same time permeable to water: their hydraulic conductivity is considerably higher than that of natural cohesive soils (Figure 6). Therefore, such layers act as permeable insulating barriers, which halt sulfide oxidation, but do not affect hydrogeological conditions in a dump or other facility constructed of mining waste.

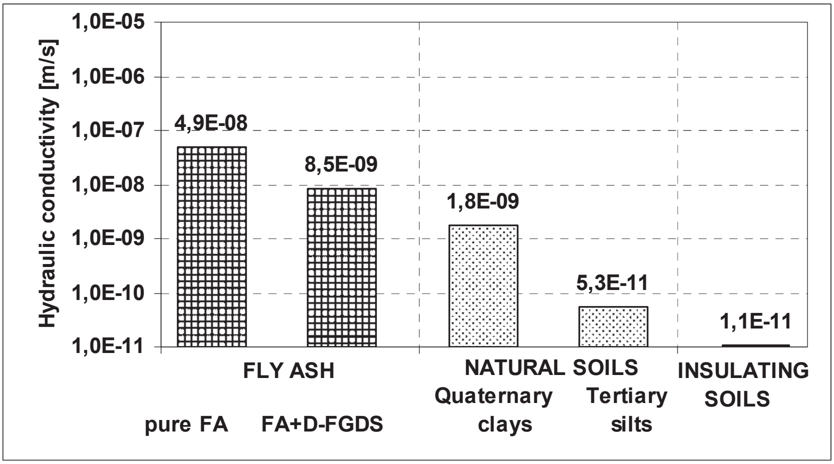

Figure 6. Hydraulic conductivity of the solidified dense FA: water mixtures (1:1 wt.) vs. that of natural cohesive soils.

Sulfide oxidation may occur in the uppermost layer of a dumping site that cannot be surficially insulated with dense FA mixtures due to the need of maintaining a vegetative cover, potential to easy damage and weathering processes. In this case, introduction of a layer comprising organogenic waste sorbent in the bottom of a vegetation-supporting mining waste cover and over the subsurface drainage system may successfully attenuate vertical migration of heavy metals generated in sulfide oxidation processes and transport with an infiltrating water stream.

5. CONCLUSIONS

The presented data illustrate opportunities of utilizing organogenic and mineral wastes, that also have high contents of hazardous elements or substances, for diverse environmental protection purposes, provided that the way of utilization complies with properties and environmental behavior of waste.

Both NOM and sewage sludge from WWTPs displayed high potential for heavy metals removal from polymetallic wastewaters from small enterprises of pH much lower than pH 3.0 considered critical for sorption to occur. The flow-through system showed substantially higher sorption capacity and efficiency than the batch one, though due to the construction and operational simplicity the latter might be better applicable in small industries.

Very good results of sewage sludge use as a sorbent in simple batch reactors for source control of heavy metals in unaltered acidic waste effluents from artisan enterprises in order to reduce heavy metal content in wastewater entering WWTPs and subsequently in sewage sludge generated in the wastewater treatment process and intended to be used in agriculture, seems to be a promising solution aimed to protection of soil quality.

Another prospective application of stabilized sewage sludge as permeable reactive layers for interception of heavy metals in leachate from sulfidic mine waste dumps, in combination with permeable insulation layers of solidified dense FA: water mixtures to prevent sulfide oxidation shows that an acceptable level of environmental protection might be accomplished with wide use of waste materials and not necessarily be expensive.

In all environmentally-related decisions where waste application is involved, issues that should be considered as the first priority are the precautionary principle and protection of natural resources.

ACKNOWLEDGEMENTS

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MEDIATING EFFECTS OF HUMIC SUBSTANCES IN THE CONTAMINATED ENVIRONMENTS

Concepts, Results, and Prospects

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Abstract: A new concept for the mediating action of humic substances (HS) in the contaminated environment is developed. It defines three scenarios of mitigating activity of HS in the system "living cell-ecotoxicant". The first scenario refers to deactivation of ecotoxicants (ET) by HS due to formation of non-toxic and non-bioavailable complexes. It takes place outside of the cell and is defined as “exterior effects”. The second scenario refers to deactivation of ET due to HS adsorption onto the cell wall or membrane and is defined as "boundary effects": sorption takes place on the cell surface and implies changes in permeability and structure of the cell membrane. The third scenario refers to amelioration of contaminant toxicity due to activation of systemic resistance to chemical stress. This implies HS participation in immune response activation and is defined as "interior" effects. Viability of this concept was confirmed by the results of detoxification experiments. It was shown that chemical binding ("exterior effects") played a key role in ameliorating toxicity of ecotoxicants (Hg(II) and PAHs) strongly interacting with HS, whereas enhanced immune response ("boundary and interior" effects) was much more operative for a decrease in toxicity of atrazine weakly interacting with HS. The formulated concept provided satisfactory explanations for a vast pool of reported findings of mitigating activity of HS reviewed in the chapter. Few cases of amplified toxicity reported for weakly interacting contaminants in the presence of low molecular weight HS were related to facilitated penetration and follow up dissociation of humic-contaminant complexes in the cell interior. It is concluded that the developed concept can be used as a prospective tool for both predictive modelling of

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mediating effects of HS in the contaminated environments and designing new humic material suitable for a use as detoxicants and plant activators.

Key words: humic substances; mercury; polycyclic aromatic hydrocarbons; atrazine; detoxifying ability; mediating effects

1. INTRODUCTION

Humic substances (HS) compose from 60 to 80% of non-living organic matter in both soil and water ecosystems (Thurman and Malcolm, 1981). With the turnover of 2 Gt of carbon per year, humification is the second largest process after photosynthesis (20 Gt/a) contributing into the global carbon cycle (Hongve et al., 1981). HS are products of chemical-microbiological synthesis occurring during decomposition of mortal remains of living organisms. In contrast to the synthesis of biopolymers in living organisms, formation of humic molecules does not have a genetic code and proceeds stochastically: the only structures that survive are those which resist further microbial and chemical decomposition. As a result, intrinsic features of HS are non-stoichiometric elemental compositions, irregular structures, heterogeneous structural units, and polydisperse molecular weights (Kleinhempel, 1970). Evidently, there are no two identical molecules of HS. Despite that, HS of different origin have a very similar structural organization. A humic macromolecule consists of an aromatic core highly substituted with functional groups (among those dominant oxygen functionalities – carboxyls, hydroxyls, carbonyls) and of peripheral aliphatic units composed mostly of polysaccharidic and polypeptidic chains, terpenoids, etc. (Stevenson, 1994).

Complex structure provides for a very diverse reactivity of HS. They are able of ionic, donor-acceptor (including hydrogen bonding and charge-transfer complexes) and hydrophobic bonding. As a result, they can bind heavy metals as well as polar and highly hydrophobic organic compounds released into environment (e.g., pesticides, polycyclic aromatic hydrocarbons, polychlorinated compounds). The mitigating impact of HS prescribed to formation of non-toxic and non-bioavailable humic complexes was numerously reported in the literature (Giesy et al., 1983; Vymazal, 1984; Landrum et al., 1985; McCarthy et al., 1985; Morehead et al., 1986; Oris et al., 1990; Perminova et al., 1999; Misra et al., 2000). On the other side, hydrophobic aromatic core and hydrophilic peripheral moieties (e.g., polysaccharides) determine amphiphylic character and surface activity of humics. This brings about biological activity of HS resulting from direct interactions with living organisms through adsorption on cell surface or by penetration into the cell (Müller–Wegener, 1988; Kulikova et al., 2005). These chemical-biological interactions provide for increasing interest to remedial uses of humic materials (Perminova and Hatfield, 2005).

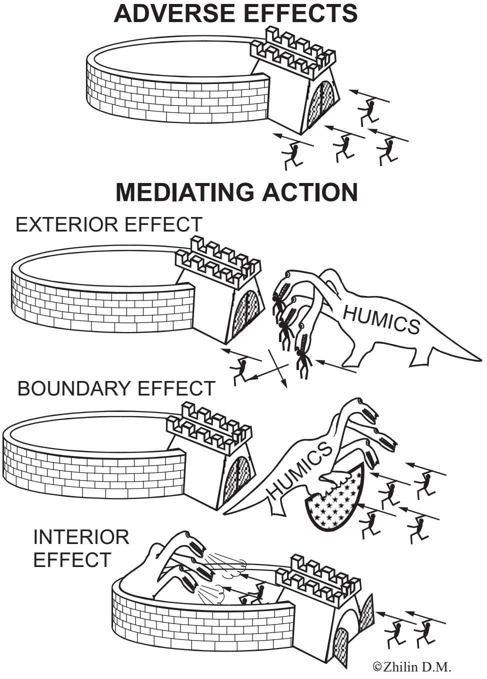

Figure 1. Conceptual model of mediating effects of HS in the system "living cell-ecotoxicant". Three proposed scenarios refer to "exterior", "boundary", and "interior" effects according to interactions involved. "Exterior effects" imply deactivation of ecotoxicant by HS due to formation of non-toxic complexes and take place outside of the cell. "Boundary effects" imply deactivation of ecotoxicant due to HS adsorption onto cell wall and take place on cell surface. "Interior effects" imply amelioration of contaminant toxicity due to activation of systemic resistance to chemical stress and take place inside of the cell due to HS participation in activating cell immune response.

The primary goal of the present paper is to develop a holistic concept of the mediating action of HS accommodating both chemical and biological interactions occurring in contaminated systems. It defines three major scenarios of mitigating activity of HS in the system "living cell-ecotoxicant", which are visualized in Figure 1 using the allegory of "dragon" (humics) taking three different tactics to protect its "fortress" (cell) from the attacking enemy (ecotoxicant). The first scenario refers to deactivation of ET due to chemical binding to HS leading to formation of non-toxic and non-bioavailable complexes. It takes place outside of the cell and is defined as “exterior effects”. The second scenario refers to deactivation of ET due to HS adsorption onto cell wall or membrane and is defined as "boundary effects": sorption takes place on cell surface and implies changes in permeability and structure of cell membrane. The third scenario refers to amelioration of ET toxicity due to activation of systemic resistance to chemical stress. This implies HS participation in immune response activation and is defined as "interior" effects.

The mediating action of HS in reality might imply simultaneous involvement of all three mechanisms. However, their prevalence will depend on molecular properties of HS. So, chemical binding is the most probable way of mediating action of HS possessing high affinity for ET. For high molecular weight HS, which cannot penetrate cell walls because of steric hindrances, this can be the only mechanism of mediating action. The “boundary” mechanism is expected to be most operative for the hydrophobic HS. The third mechanism can be of particular importance for low molecular weight fractions of HS, which can penetrate the cells.

2. REVIEW OF MEDIATING EFFECTS OF HUMIC SUBSTANCES ON SELECTED CONTAMINANTS

2.1 Mercury

An overview of the effects exerted by HS upon mercury toxicity to and bioaccumulation by the aquatic test organisms demonstrates a purely mitigating action of HS regardless of its source and a type of the test-organisms. Decrease in mercury toxicity and bioaccumulation in the presence of natural and synthetic organic chelating agents is widely reported in literature (Oikari et al., 1992; Sjoblom et al., 2000; Hammock et al., 2003) and usually is related to a reduction in concentration of Hg(II).

2.2 Polycyclic Aromatic and Polychlorinated Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated dioxins (PCDDs) and polychlorinated biphenyls (PCBs) belong to the large class of hydrophobic organic contaminants (HOC). Both uptake and bioaccumulation of these compounds are strongly dependent on hydrophobicity of the chemical (as indicated by its octanol/water partition coefficient, K_{OW}) (Hawker and Cornell, 1985; Akkanen and Kukkonen, 2003) and environmental factors which can modify its physical-chemical state (Müller–Wegener, 1988). Among the factors determining speciation of HOC in aquatic systems, the most important is their partitioning to particulate and dissolved organic matter (Carlberg and Martinsen, 1982; Landrum et al., 1985; Chiou et al., 1987; Hur and Schlautman, 2004). The importance of this process for toxicity and bioavailability of PAHs, PCDDs and PCBs is demonstrated by numerous publications (Landrum et al., 1985; Carlberg et al., 1986; McCarthy and Bartell, 1988; Servos et al., 1989; Oikari and Kukkonen, 1990; Weber and Lanno, 2000; Reid et al., 2001). Hydrophobic binding is assumed to be a major mechanism of HOC association with DOM (Merkelbach et al., 1993; Perminova et al., 1999). Given that DOM in natural waters is predominantly composed of HS (about 50% of DOC (Thurman and Malcolm, 1981) many publications pay specific attention to chemical interaction between HS and HOC. It is found that humics – HOC association is the stronger, the higher K_{OW} both for humic and HOC compounds are (McCarthy et al., 1989; Merkelbach et al., 1993; Kopinke et al., 1995; Poerschmann et al., 2000). On the other hand, HOC sorption is greatly affected by chemical structure and composition of HS (Kang and Xing, 2005). Taken into consideration that HS hydrophobicity is determined by their structure (Kopinke et al., 1995), the binding constant for the same contaminant can greatly vary depending on the source of humics. For example, the most hydrophobic Aldrich HA displayed the highest affinity for HOC, whereas the most hydrophilic HS – surface water DOM were characterized with the lowest affinity for HOC (Landrum et al., 1985; Morehead et al., 1986).

Binding to HS controls accumulation of HOC by aquatic organisms. This is solidly confirmed by the results on HOC bioaccumulation from humics-containing solutions. All humic materials, regardless of their origin, caused a reduction in bioaccumulation of PAHs, PCBs and TCDD both by crustaceans and fish species. The reduction was proportional to hydrophobicity of HS present and generally consistent with the value of binding constants. Fractionation of DOM with a use of XAD-8 resin showed that it was hydrophobic anionic fraction of the DOM, which was responsible for the contaminant-DOM interaction (McCarthy et al., 1989; Kukkonen, 1991). Exceptions from the given observations were low hydrophobic PAHs (naphthalene, anthracene, phenanthrene) and extremely hydrophobic congeners of PCDD with more than six substituting chlorine atoms whose bioaccumulation was practically not affected by HS (Bruggeman et al., 1984). The above results brought different researchers to a conclusion that a use of the commercially available Aldrich HA as a model humic material can greatly overestimate the potential of DOM to reduce HOC bioaccumulation by aquatic biota. It means that particular caution should be exercised while predicting mitigating impact of HS on bioaccumulation potential of PAHs and polychlorinated hydrocarbons in a real aquatic system. It is especially true given the fact, that influence of HS on toxicity of these chemicals is hardly studied at all.

2.3 Pesticides and Other Organic Xenobiotics

Another big group of hazardous organic chemicals is presented by pesticides, which vary greatly in their chemical structure. All pesticides are characterized by high toxicity to biota (Choudhry, 1983; Draber et al., 1991). A whole number of publications are devoted to examination of pesticide toxicity in the presence of HS (Stewart, 1984; Day, 1991; Oikari et al., 1992; Genevini et al., 1994; Steinberg et al., 1994; Freidig et al., 1997; Gensemer et al., 1998; Koukal et al., 2003; Mézin and Hale, 2003). However, there is a substantial disparity in the reported findings. This can be explained by diverse chemical and physiological mechanisms involved. Comparison of the HS effects exerted on toxicity of pesticides, substituted phenols and anilines shows that enhanced toxicity was mostly observed in the test solutions containing low molecular weight HS. For example, increase in acute toxicity of nine out of thirteen organic chemicals was found in the presence of DOM from different lakes (Oikari et al., 1992). Stewart (1984) reported on enhanced toxicity of eight methylated phenols and anilines to algae in the test solution containing commercially available soil fulvic acids with molecular weight ranging from 643 to 951. Comparison of HS effects of different origin – Aldrich HA (Na-form) and DOM – on toxicity of fenvalerate (Oikari et al., 1992) – shows that in the presence of high molecular weight hydrophobic Aldrich HA a reduction in toxicity takes place, whereas enhanced toxicity of fenvalerate is observed in the DOM-containing test solution. Very similar results were reported by Loffredo et al. (Loffredo et al., 1997). The authors observed a reduction in toxicity of three herbicides in the presence of soil HA, whereas sewage sludge HA (which are likely of lower molecular weight) resulted in an increased toxicity.

Summarizing the above data, it can be concluded that sensibilizing effect of low molecular weight fractions of HS on toxicity of some trace metals also takes place in case of pesticides and substituted phenols/anilines. These effects can be linked to long-term observations of soil scientists on the specific physiological action of low molecular weight compartment of soil humics. According to numerous studies (Prát, 1963; Batalkin et al., 1982; Samson and Visser, 1989; Mazhul et al., 1993; Ermakov et al., 2000), HS can permeate or modify cell membranes. It is shown also, that FA are to be taken up to a larger degree than HA, and lower molecular weight HS (< 2500 D) to a greater extent than the higher molecular weight material (Prát, 1963; Führ, 1969; Führ and Sauerbeck, 1965). This allows a suggestion that membranotropic fraction of HS can facilitate penetration of bound to it low molecular weight compounds into the cell. These compounds can be heavy metals ions as well as molecules of pesticides or other toxic chemicals. It is the enhanced translocation of the toxic chemicals across biological membranes, which seems to be responsible for the discussed above increase in toxicity of some contaminants in the presence of HS with lower molecular weights.

The given contradictory findings show an importance of systematic studies on mediating effects of HS in the contaminated environments, which would couple chemical and toxicological interactions between ET and HS in the framework of a holistic conceptual model of the mediating action of HS.

3. CONCEPTUAL MODEL OF MEDIATING ACTION OF HS

To estimate contribution of chemical binding in mediating action of HS, an original approach has been undertaken described in detail in our previous publication (Perminova et al., 2001). According to this approach, interaction between HS and ET can be schematically described by the following equation:

$$\text{HS} + \text{ET} \leftrightarrow \text{HS-ET} \tag{1}$$

To quantify this interaction, an equilibrium constant K is commonly used:

$$K = \frac{[\text{HS-ET}]}{[\text{ET}] \times [\text{HS}]} \tag{2}$$

where [HS], [ET] and [HS-ET] are the equilibrium concentrations of the reagents and reaction product.

Given that the total HS concentration (C_{HS}) in natural system is much higher than that of ET (C_{ET}), [HS] in Eq. (1) can be substituted with C_{HS}. Because of the unknown stoichiometry of the reaction, C_{HS} is usually expressed on a mass rather than on a molar basis (Perminova , 1998; Swift, 1999). Due to that, the binding constant K can be rewritten in the form of sorption constant K_{OC} as follows:

$$K_{OC} = \frac{1-\alpha}{\alpha} \times \frac{1}{C_{HS}} \qquad (3)$$

where α is the portion of freely dissolved ET in the presence of HS, $\alpha = [\mathrm{ET}]/C_{ET}$, and C_{HS} is the total mass concentration of HS normalized to the content of organic carbon (OC), kgC/L.

The above expression allows us to estimate binding affinity of HS for ET by determining a portion of freely dissolved ET in the presence of HS. This can be done using common analytical techniques with or without preliminary separation of freely dissolved and HS-bound species of ET. On the other side, assuming that only freely dissolved ET is biologically active (toxic and bioavailable), the same binding constant can be determined from reduction in toxicity (T_{ET}) or bioaccumulation (BCF_{ET}) in the presence of HS. If the assumption were valid, binding constants determined from a reduction in concentration of freely dissolved ET using analytical techniques and from a reduction in toxicity and bioaccumulation using bioassay techniques would be equal. Thus, the similarity of the binding constants determined from chemical and toxicological experiments can be used for making a judgment on the contribution of chemical binding into mediating action of HS. Indeed, if only freely dissolved ET is toxic, then toxicity T in the presence of HS (T_{ET+HS}) can be expressed as follows:

$$T_{ET+HS} = \alpha \bullet T_{ET} \qquad (4)$$

In this case, detoxification effect D exerted by HS can be defined as:

$$D = (T_{ET} - T_{ET+HS})/T_{ET} \qquad (5)$$

If toxicity of ET is proportional to its concentration in the test system:

$$T_{ET} = k \bullet C_{ET} \text{ and } T_{HS+ET} = k \bullet [\mathrm{ET}] \qquad (6)$$

then D is numerically equal to a portion of ET bound to HS, $(1 - \alpha)$. In this case, dependence of D on concentration of HS can be described by "detoxification" constant (K_{OC}^{D}) with a use of Eq. (3) as follows:

$$D = \frac{K_{oc}^{D} \times C_{HS}}{1 + K_{oc}^{D} \times C_{HS}} \tag{7}$$

The corresponding K_{oc}^{D} constant is an analogue of binding constant K_{oc} and can be calculated by fitting experimental relationships of D *versus* C_{HS} with a use of non-linear regression. To determine D from experimental data, ET toxicities in the absence and presence of HS were defined as follows:

$$T_{ET} = \frac{R_0 - R_{ET}}{R_0} \tag{8}$$

$$T_{ET+HS} = \frac{R_{HS} - R_{HS+ET}}{R_{HS}} \tag{9}$$

where R_0 is the response of a test organism in control solution, R_{ET} is the response of a test-organism in the ET solution, R_{HS} is the response of a test-organism in the HS solution; R_{HS+ET} is the response of a test-organism in the solution of ET with HS present.

To determine a reduction in ET toxicity provided exclusively by chemical binding to HS, its toxicity in the presence of HS (T_{ET+HS}) was normalized not to the control, but to the response of a test-organism in the presence of HS. The purpose was to take into account a possible stimulation effect of HS, while a decrease in ET toxicity in the presence of HS reflects a combined action of two effects: first, *toxicity sequestration* caused by a reduction in the concentration of freely dissolved ET due to binding to HS; and second, *stimulating effect* of HS on the test organism.

The corresponding detoxification effect D can be defined as follows:

$$D = \left(1 - \frac{R_{HS} - R_{HS+ET}}{R_{HS}} \Big/ \frac{R_0 - R_{ET}}{R_0}\right) \times 100\% \tag{10}$$

It means that each experimental point on detoxification curve results from four measurements of different responses of the target organism. To ensure validity of the given model for toxicity data, it should be additionally assumed that sensitivity of test-organisms both to toxic action of freely dissolved ET and direct effect of HS does not change under impact of HS and ET present in the test system, respectively.

Bioconcentration factor (*BCF*) of ET can be defined with a use of the following expression:

$$BCF_{ET} = \frac{\text{concentration of ET in test - organism } (\mu\text{g/g w/w})}{\text{concentration of ET in the test solution } (\mu\text{g/ml})} \tag{11}$$

In contrast to toxicity, BCF is not strongly affected by direct effect of HS, and depends mostly on the equilibrium concentration of ET in the presence of HS. It can be calculated with a use of the following expression:

$$BCF_{ET+HS} = \frac{\text{concentration of ET in test - organism } (\mu\text{g/g w/w})}{\text{equilibrium concentration of ET in the presence of HS } (\mu\text{g/ml})} \tag{12}$$

In this case, the portion of freely dissolved ET can be written as follows:

$$\alpha = \frac{BCF_{ET+HS}}{BCF^{0}_{ET}} \tag{13}$$

The corresponding “bioaccumulation” constant (K^B) can be determined by fitting an experimental dependence of a reduction in *BCF* equal to the portion of ET bound to HS *(1-α)* versus concentration of HS by the model yielding from Eq. (3):

$$B = \frac{K^{B}_{oc} \times C_{HS}}{1 + K^{B}_{oc} \times C_{HS}} \tag{14}$$

To test validity of the proposed approach, a corresponding experimental set up was developed. It implied determination of ET binding constants using both analytical and biotesting techniques. To encompass as broad range of mediating effects of HS as possible, three classes of ET greatly differing in chemical properties and biological activity were used for our studies, nominally: Hg(II) (the most toxic heavy metal), PAH – pyrene, fluoranthene, anthracene (highly hydrophobic organic contaminants) and atrazine (sim-triazine herbicide, specific inhibitor of photosynthesis). Of particular importance is, that all studies were conducted using representative sets of structurally diverse HS samples from main natural sources (coal, peat, soil, fresh water) and of different fractional composition (humic *versus* fulvic acids). To assure statistical significance of the obtained results, each experimental set accounted for 20-25 HS samples.

4. EXPERIMENTAL PART

4.1 Ecotoxicants

For all experiments, *mercury(II)* salts of pure grade were used. The concentrations of mercury were $0.8\cdot10^{-6}$ M (as $HgCl_2$) and $(0.1–0.8)\cdot10^{-6}$M (as $Hg(NO_3)_2$) for toxicity tests and analytical techniques, respectively.

PAHs used were *anthracene* (Aldrich, 98% pure), *fluoranthene* (Aldrich, 97% pure), *and pyrene* (Aldrich, 97% pure). The batch technique described elsewhere (Perminova et al., 1999) was used for preparation of aqueous solutions of the selected PAHs. For toxicity tests, the concentrations were $1.7\cdot10^{-7}$, $7\cdot10^{-7}$ and $5\cdot10^{-7}$M for anthracene, fluoranthene and pyrene, respectively.

Atrazine (99.97%) was purchased from Dr. Ehrensdorf Ltd. A stock solution of atrazine ($4.6\cdot10^{-5}$M) was prepared in distilled water and stored in the dark at 4 °C.

4.2 Humic Materials

Humic materials used were isolated from different natural sources (fresh water, soil, peat) using techniques described below.

Aquatic humic materials were isolated with a use of sorption on XAD-2 resin and follow-up elution with 0.1 M NaOH (Mantoura and Riley, 1975). The alkali extracts were desalted and used without further fractionation as a mixture of FA and HA. They were designated as *AHF*. Samples of native swamp water served as preparations of the aquatic dissolved organic matter (*ADOM*). *Peat humic materials* were isolated from 5 highland and 4 lowland peats of different geobotanical composition. The isolation procedure was as described elsewhere (Lowe, 1992) and included a preliminary treatment of a peat sample with an ethanol-benzene (1:1) mixture followed up by an alkaline (0.1 M NaOH) extraction. The alkali extracts were desalted and used without further fractionation as a mixture of FA and HA. They were designated as *PHF*. One sample was a concentrated water extract of woodyherbaceous peat (*PDOM*). *Soil humic acids (SHA)* were extracted from soils of different climatic zones: Sod-podsolic soils (Moscow and Novgorod regions), gray wooded soils (Tula region), chernozems (Voronezh region). Sod-podzolic soils were also of various agricultural uses: virgin, plough, garden. The HS extraction was carried out according to Orlov and Grishina (Orlov and Grishina, 1981). This included pre-treatment of a soil sample with 0.1 M H_2SO_4, follow up alkaline extraction (0.1 M NaOH), and acidification of the extract to pH 1-2. The precipitated HA were desalted by dialysis. *Soil fulvic acids (SFA)* were extracted from 3 sod-podzolic soils of different agricultural use, virgin grey wooded soil and typical chernozem. To isolate FA, the supernatant after precipitation of HA was passed through Amberlite XAD-2 resin. Further treatment was as described for aquatic HS. *Non-fractionated mixtures of HA and FA* of soil (*SHF*) and fresh water bottom sediments (*BHF*) were isolated by alkaline extraction (0.1 M NaOH) of soil or bottom sediment sample without further fractionation of the extract. To isolate *non-fractionated mixtures of water-soluble soil HA and FA* (*SDHF*), the acidified (pH 1–2) water extract (1:2) of three sod-podzolic soils (virgin, plough, and garden) was passed through Amberlite XAD-2 resin and follow up elution with 0.1 M NaOH. Then the alkali extracts were desalted. Commercial samples of *coal HA* (*CHA*) – *Aldrich Humic Acid* (*CHA-AHA*) and *Activated Coal Humic Acid* (*CHA-AGK*) (Biotechnology Ltd., Moscow, Russia) were used as purchased from the suppliers. Concentrated stock solutions of HS (100–500 mgOC/L) were prepared by evaporation of the corresponding desalinated isolates or by dissolution of a weight of a dried material.

Structural characterization of HS. The target humic materials were characterised with the data of elemental analysis, size-exclusion chromatography (SEC) and ^{13}C NMR spectroscopy. The corresponding characteristics are given in our previous publications (Perminova et al., 1999, 2001). In brief, the humic materials used were characterized with the following parameters: contents of elements and atomic ratios (C, H, N, O, H/C, O/C), molecular weight, molar absorptivity at 280 nm (ABS_{280}) and contents of carbon in the main structural groups as measured using ^{13}C NMR under quantitative conditions (Kovalevskii et al., 2000). The assignments in the ^{13}C NMR spectra were made after (Kovalevskii et al., 2000) and were as follows (in ppm): 5-50 – aliphatic H and C-substituted C atoms (C_{Alk}), 50-108 – aliphatic O-substituted C atoms (C_{Alk-O}), 108-145 – aromatic H and C-substituted atoms ($C_{Ar-H,C}$), 145-165 – aromatic O-substituted C-atoms (C_{Ar-O}), 165-187 – C atoms of carboxylic and esteric groups (C_{COO}), 187-220 – C atoms of quinonic and ketonic groups ($C_{C=O}$).

For the experiments with *Hg(II)*, a set of 24 humic materials was used including 2 ADOM, 5 AHF, 3 BHF, 6 PHF, 3 SHA, 3 SHF and 2 CHA. For the experiments with *PAH* a set of 26 humic materials was used including 4 AHF, 7 PHF, 1 PDOM, 8 SHA and 5 SFA. For the experiments with *atrazine*, a set of 25 humic materials was used including 1 AHF, 3 PHF, 2 PDOM, 9 SHA, 5 SFA, 1 SHF and 3 SDHF.

4.3 Determination of the Hinding Constants Using Analytical Techniques

Mercury. Chemical binding of Hg(II) to humic materials was quantitatively characterized with an amount of the mercury-binding sites (BS) in the humic material and with the values of the stability constants of the Hg-HS complexes. To determine an amount of the BS in humic materials, the saturated Hg-HS precipitates were obtained at pH 2 under conditions described in (Zhilin et al., 1996). The content of Hg in the saturated humates (meq/g) was treated as equal to the BS content in the humic material. Basing on this parameter, Hg-equivalent concentration of humic preparation was determined and used for calculation of the stability constant of Hg-HS complexes instead of the molar concentration of HS. To determine the corresponding stability constant K_{BS}, ligand exchange technique with a use of adsorption was applied (Yudov et al., 2005). For this purpose, Hg(II) adsorption on polyethylene surface from 0.0025 M hydrocarbonate buffer was studied in the presence of HS. Total Hg(II) concentration accounted for 50–250 nM, concentration of HS 2-40 mg/L, pH 6.9–7.2. Hg(II) concentration in the presence of HS was determined using cold vapor AAS technique as described in (Zhilin et al., 2000).

PAH. Binding of PAH to dissolved humic materials was characterized by partition coefficients determined with a use of fluorescence quenching technique ($K_{\mathrm{OC}}{}^{fq}$) as described in (Perminova et al., 1999). The PAH solutions below the water solubility limit (0.6×10^{-7}, 1×10^{-7} and 5×10^{-7} M for pyrene, fluoranthene and anthracene, respectively) were prepared by the solubilisation technique. The concentration of HS was in the range of $(0.2\text{-}6)\times10^{-6}$ kgC/L. The slopes of the obtained Stern-Volmer plots yielded the $K_{\mathrm{OC}}{}^{fq}$ values.

Atrazine. Binding of atrazine to humic materials was characterized with the binding constant K_{OC}. Binding experiments were conducted at pH 5.5 as described in (Kulikova and Perminova, 2002). The concentration of HS was in the range of $(0.2–0.8)\times10^{-3}$ kgC/L, initial concentration of atrazine was $9.3\cdot10^{-6}$ M. For the separation of the freely dissolved and the bound to HS fractions of herbicide, the batch ultrafiltration technique was applied using a membrane filter with a molecular weight cut-off of 1000 D. Atrazine was determined in the ultrafiltrate with a use of a HPLC technique.

4.4 Toxicity Tests

Depending on ET type and media (aquatic or soil), different test organisms were chosen to carry out toxicity tests. Toxicity tests in aquatic media were conducted using as target organisms green algae *Chlorella* (Hg and atrazine) and Crustacea *Daphnia* (PAH). For soil bioassays with atrazine, wheat plants were used.

According to the proposed approach, each experimental series included the following treatments: control (water or nutrient solution), ET solution at the working concentration, HS solutions at five different concentrations, solutions of ET+HS at the working concentration of ET and above concentrations of HS. The obtained data were used for calculation of *D* using Eq. (10) at each concentration of HS.

Toxicity tests with algae. For the toxicity tests with Hg and atrazine, green algae *Chlorella pyrenoidosa* was used as described in (Polynov, 1992). The relative yield of variable fluorescence F_v/F_m was used as a target response. It is indicative of the quantum yield of chlorophyll fluorescence and can be derived from the induction fluorescence curve (Kautski effect). The algae were cultured in 20% nutrient solution (Tamiya et al., 1961) in temperature-controlled vessels at 35±2 °C under continuous irradiance of 60 μmol photons/m^{2}s provided by fluorescent lamps and bubbling with moisturized air. Prior to toxicity tests, algal cells were concentrated by centrifugation and resuspended in 10% Tamiya nutrient solution without phosphate or EDTA to yield an initial concentration of about 10^6 cells/ml. Then ET or HS or their mixture was added to algae cells. F_v/F_m ratio was determined after 4 (in case of Hg) or 3 hours (in case of atrazine) exposure under the same conditions as for algae growing. Fluorescence induction curve parameters (F_v/F_m and F_i/F_m) of algae chlorophyll were calculated after subtraction of the self-fluorescence of humic materials.

$HgCl_2$ was used as a toxic Hg(II)-species for conducting toxicity tests. The working concentration of $HgCl_2$ was $0.8 \cdot 10^{-6}$ M; concentration of HS varied in the range of 2.5–12.5 mg C/L; pH 7.2. Toxicity tests with atrazine were conducted at its working concentration of $6.7 \cdot 10^{-7}$ M. The concentration of HS varied in the range of 0.6-5 mg C/L; pH 6.8.

Toxicity tests with Daphnia. Acute toxicity tests with PAHs were performed according to Perminova et al. (2001). The Crustacea *Daphnia magna* was used as a test organism, its grazing activity (averaged filtration rate per *Daphnia*, ml/hr) – as a target response. Daphnids were cultured in tap water at 20 °C with a light: dark rhythm of 16 h:8 h. A suspension of the green algae *Chlorella vulgaris* was used to feed *D. magna* daily. For the toxicity tests, 5–6 day old animals were used. The working solutions of PAH were prepared at the maximum achievable concentrations of, nominally, $5 \cdot 10^{-7}$, $7 \cdot 10^{-7}$, and $1.7 \cdot 10^{-7}$ M for pyrene, fluoranthene and anthracene, respectively. The concentration of HS varied in the range from 1.5 to 25 mg C/L. Three replicates were made for each assay.

Toxicity tests with plants. Three sod-podzolic soils of different agricultural use (garden, plough, and virgin) were used for testing. The commercial preparation of brown coal HA (AGK) was used as a detoxifying agent for soil tests. Wheat plants *Triticum aestivum* served as a test-object and wet biomass of plants as a target response. The application rate of atrazine was 1 mg/kg, the rate of CHA-AGK ranged from 35 to 105 mg/kg. The plants were harvested after 30 days of growth. Calculation of the detoxification coefficient D and binding constant K_{OC}^{D} was performed as described above.

5. RESULTS AND DISCUSSION

5.1 Aquatic Media

Typical dependencies of Hg, PAHs, and atrazine toxicities upon HS concentration in the test system are given in Figure 2. Toxicity of all model ET decreased with an increase in HS concentration. Detailed explanations of the results obtained for each ET are presented below.

Mercury. The bioassays with $HgCl_2$ implied both bioaccumulation and toxicity measurements in the presence of HS. Both toxicity and bioaccumulation of $HgCl_2$ decreased along with an increase in HS concentration (Figure 2). This is in agreement with the findings of other investigators (Oh et al., 1986).

From the data on toxicity and *BCF* changes, the detoxification (K_{BS}^{D}) and bioaccumulation (K_{BS}^{B}) constants of Hg-HS complexes were calculated as described above. For this purpose, the Hg-equivalent concentration of HS was estimated (Zhilin, 1998). To calculate the K_{BS}^{D}, it was assumed that Hg(II) was distributed between $HgCl_2$ and Hg-BS complexes (BS is a binding site of a humic molecule). This allowed us to express K_{BS}^{D} as follows:

$$K_{BS}^{D} = K_{BS/Cl} \bullet K_{HgCl_2} \tag{15}$$

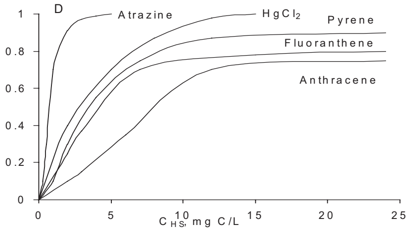

Figure 2. Typical detoxification curves - relationships of detoxification coefficient D versus HS concentration for different model ecotoxicants: PAHs, Hg(II), and atrazine.

where $K_{BS/Cl}$ is the constant of ligand exchange, K_{HgCl2} is stability constant of $HgCl_2$.

K_{BS}^{D} values determined at I = 0.019M (ionic strength of the cultivation medium) were then adjusted to I = 0.0 M using the Davies equation. The log K_{BS}^{D} and log K_{BS}^{B} values laid in the range of 13.0–14.4 and 13.0–15.3, respectively. These were on the same order of magnitude as K_{BS} values determined using analytical technique. Of particular interest was the statistically significant correlation (P>95%) found between K_{BS}^{D}, K_{BS}^{B} and log K_{BS}. The *r* values were 0.64 and 0.69 for the pairs of variables "K_{BS}^{D}-log K_{BS} " and "K_{BS}^{B}-log K_{BS}", respectively. According to the criteria set for the proposed approach, a conclusion was made that the main mechanism of $HgCl_2$ toxicity and bioconcentration sequestration by HS is formation of non-toxic and non-bioavailable Hg-HS complexes.

PAH. Typical relationships of PAH toxicities versus HS concentration are given in Figure 2. The observed toxicities decreased along with an increase in HS concentration. These effects were observed for all humic materials except for soil FA that did not influence PAH toxicities. The calculated K_{OC}^{D} values for pyrene, fluoranthene and anthracene have been reported (Perminova et al., 2001). Among PAHs studied, comparable K_{OC}^{D} values were obtained for pyrene ($1.1 \cdot 10^5$–$6.0 \cdot 10^5$ L/kg C) and fluoranthene ($0.4 \cdot 10^5$–$8.0 \cdot 10^5$ L/kg C), and the lower ones were observed for anthracene ($0.3 \cdot 10^5$–$6.0 \cdot 10^5$ L/kg C). This can be related to lower hydrophobicity of anthracene (log K_{ow} = 4.45) compared to pyrene and fluoranthene (log K_{ow} = 4.88 and 5.16, respectively) (Hansch et al., 1995). Similar trends were previously reported for PAH bioconcentration in the presence of HS (Steinberg et al., 2000).

Based on the measured K_{OC}^{D} values, the target humic materials can be arranged in the following descending order: Aldrich HA ≅ chernozemic HA>> sod-podzolic and grey wooded soil HA ≅ peat HF >> aquatic HF ≅ chernozemic FA >> sod-podzolic and grey wooded soil FA. These trends with the source of HS are in agreement with those observed in our previous studies on partition coefficients determined with a use of the fluorescent quenching technique (K_{OC}^{fq}) (Perminova et al., 1999). They are also consistent with the findings of other investigators. For example, very high binding affinity of Aldrich HA was reported in (McCarthy et al., 1989; Chin et al., 1997). Higher K_{OC} values for soil HA than for soil FA were reported in (Gauthier et al., 1987; De Paolis and Kukkonen, 1997).

Statistical data treatment showed rather strong positive correlation between K_{OC}^{D} and K_{OC}^{fq} (r = 0.86, 0.85, and 0.76 for pyrene, fluoranthene and anthracene, respectively). Intercepts of regression lines were equal to zero and slopes – significantly different from one (mean±CI, P = 95%):

$$\text{pyrene: } K_{OC}^{D} = (2.6 \pm 0.3) \bullet K_{OC}^{fq} \qquad (16)$$

$$\text{fluoranthene: } K_{OC}^{D} = (4.6 \pm 0.6) \bullet K_{OC}^{fq} \qquad (17)$$

$$\text{anthracene: } K_{OC}^{D} = (6.0 \pm 1.4) \bullet K_{OC}^{fq} \qquad (18)$$

Thus, the $K_{OC}^{fq}s$ account for about 74, 72 and 58% of variability in the $K_{OC}^{D}s$ for pyrene, fluoranthene and anthracene, respectively. However, slopes of the regression lines indicated a constant bias between the partition coefficients estimated by two techniques used. As was shown in (Perminova et al., 2001), the observed bias between the K_{OC}^{fq}s and K_{OC}^{D}s, both of those are *conditional constants*, resulted from differences in conditions of their determination. The fluorescence quenching determinations were conducted in distilled water, whereas the toxicity tests – were in filtered tap water. The presence of Ca^{2+} and Mg^{2+} in tap water at the concentration of 4 mM seems to be the most probable reason of substantially higher K_{OC}^{fq} values in comparison with those determined in distilled water. An increase in K_{OC}^{fq} value in the presence of Ca^{2+} was reported by Schlautman and Morgan (1993) for pyrene and anthracene. Hence, the detoxification effect of HS could be mostly attributed to chemical binding, nominally, to formation of non-toxic PAH-HS complexes.

Atrazine. The typical relationship of atrazine toxicity versus HS concentration is shown in Figure 2. The toxicity decreased along with an increase in HS concentration. Among humic materials studied, HS of soil solution displayed the highest detoxifying ability; coal and chernozemic HA had comparably high detoxifying ability, whereas soil FA did not affect significantly herbicide toxicity. The calculated K_{OC}^{D} values laid in the range from $5.0 \cdot 10^4$ to $2.1 \cdot 10^6$ L/kg C being about three orders of magnitude higher than those obtained using ultrafiltration technique (87–580 L/kg C)

(Kulikova and Perminova, 2002). This means that the observed detoxification effects cannot be explained only by the chemical binding of atrazine. Of particular importance is, that there was no correlation ($r = 0.12$) found between the values of K_{OC} and K_{OC}^D.

This made us to hypothesise another mechanism for the mediating effect of HS in case of atrazine, nominally, direct stimulating impact of HS displayed under stress conditions. A similar phenomenon was numerously reported in the literature for the higher plant experiencing draught conditions, temperature stress, unfavourable pH, and others (Kulikova et al., 2005 and citations in it). To test this hypothesis, additional experiments were conducted under temperature stress conditions. For this purpose, the thermophilic stain of algae *Chlorella pyrenoidosa* was cultivated at 25 °C instead of the optimum 35 °C during three hours in the presence of HS. After three hours of incubation, photosynthetic activity of algae in the absence of HS (water soluble HS extracted from the forest SDHF1 and garden SDHF3 sod-podzolic soils were used) almost equalled zero. Increase in HS concentration was accompanied with enhanced photosynthetic activity of algae. When HS concentration reached 2 mg C/L, F_v/F_m values equalled to those recorded under optimum temperature conditions (Figure 3). The obtained data show that HS greatly stimulated photosynthetic activity of algae cells under conditions of the temperature stress. Of particular interest is that there was no stimulating activity of HS in relation to algae tested under optimum temperature conditions. Hence, it seemed to be feasible that the detoxification effect of HS is rather a consequence of the beneficial effect of HS than that of the chemical binding of atrazine to HS.

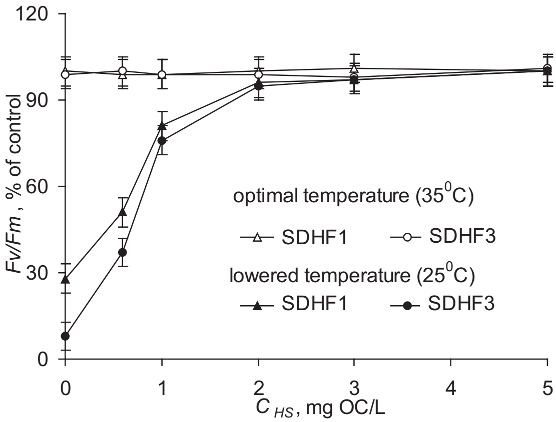

Figure 3. Dose-dependent stimulating effects of HS exerted on algae cultivated under stress conditions (lowered temperature).

As a possible mechanism of the stimulating impact of HS on living organisms under non-optimum conditions, an increase in permeability of the cell membrane could be proposed. In terms of Figure 1, we are facing here with the “boundary”, rather than with the “exterior” effects of HS. It can be surmised that HS can be used as “building material” for repairing the cell membrane under stress conditions. This can improve physiological state of the stressed algae and, as a consequence, its photosynthetic activity. To gain deeper understanding of the mechanism of detoxifying action of HS in relation to atrazine, additional studies are needed. From our point of view, the first priority should be given to studies on penetration of HS into the plant cells.

5.2 Mediating Effects of HS in the Soil System

To study mediating effects in soil, laboratory experiments with atrazine were carried out. For this purpose, soil samples were contaminated with atrazine (1 mg/kg) and mixed with different amounts of HS preparation. Three sod-podzolic soils of the different agricultural use (virgin, plough and garden) were used in these studies. Coal HA was used as a humic material. The prepared soils were further used for wheat growth experiments.

Addition of HA to all three soils resulted in a decrease in atrazine toxicity. The calculated D values varied depending on the soil type. Maximum and minimum D values of 0.52 and 0.14 were observed in virgin and plough soils, respectively. D values correlated well with actual atrazine toxicity in the soils under study. So, wet biomass of the test plants treated with atrazine was 75, 10 and 20% of the control values in the virgin, plough and garden soils, respectively. For all soils studied, an application of HA at the highest rate of 105 mg/kg led to a (50±6)% increase in the plant biomass compared to the variant with atrazine alone.

The K_{OC}^{D} values calculated from a reduction in atrazine toxicity induced by HA additions accounted for $2.1 \cdot 10^4$, $0.3 \cdot 10^4$ and $0.7 \cdot 10^4$ L/kg C for virgin, plough and garden soils, respectively. These values are considerably smaller that those obtained for dissolved HS. However, similar to dissolved HS, the obtained K_{OC}^{D} values were about two orders of magnitude higher than those obtained for atrazine binding to HS immobilized onto kaolin clay (Celis et al., 1998). The obtained results allowed us to conclude that amelioration of atrazine toxicity in soil is determined by both direct stimulating impact of HS on plants and chemical binding of atrazine to HS.

6. CONCLUSIONS

A new concept of mediating action of HS in the system living cell – ecotoxicant is developed. It defines mechanisms underlying mediating effects of HS in the context of intra- and extra-cellular processes. The first category is related to chemical binding and defined as “exterior effects”: the corresponding processes take place outside of the cell and imply formation of non-bioavailable and non-toxic HS-ET complexes. The second category is related to HS adsorption on cell walls or membranes and defined as "boundary effects": sorption takes place on cell surface and implies changes in permeability and structure of the cell membrane. The third category is related to penetration of HS alone or HS-ET complexes into the cell and defined as “interior effects”. The corresponding processes mostly take place in stressed organisms and imply participation of penetrated HS in the cell metabolism (e.g., induction of systemic acquired resistance and enhanced system immunity) or break down of the penetrated HS-ET complexes inside of the cell and increase in toxicity.

Viability of this concept was demonstrated both by reviewing literature data and by results of our own detoxification experiments. The reported findings revealed uniformly mitigating effects of humic substances on toxicity of the contaminants having high affinity for binding to HS (e.g., Hg, PAHs). For contaminants of weak or intermediate affinity for HS (e.g., chloranilines, terbutylazine), both ameliorated and amplified toxicity was observed in the presence of HS. This allowed us to suggest that chemical binding played a key role in detoxification of contaminants capable of forming stable complexes with HS, whereas direct interaction of HS with living cells including changes in membrane surface and permeability contribute the most in mediating effects exerted upon contaminants weakly bound to HS.

This suggestion was confirmed by toxicity assays conducted in the presence of HS with contaminants of different binding affinity. Strong correlation between binding affinity and detoxifying ability of HS was observed for PAHs and Hg(II), whereas no correlation with binding affinity, but highest detoxifying effects were observed for weakly interacting atrazine. Stimulating impact of HS on living cells under stress conditions was proposed as leading detoxifying mechanism in case of atrazine. This was confirmed by the results of same bioassays with alternative stress factor – temperature. As in case of atrazine presence, dose-dependent stimulating effects were observed in HS-containing test-systems cultivated under lowered temperature. The conducted studies allow a conclusion on promising applications of HS as detoxicants and plant activators in the fields of bioremediation and plant protection.

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METAL BINDING BY HUMIC SUBSTANCES AND DISSOLVED ORGANIC MATTER DERIVED FROM COMPOST

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Abstract: Composting of MSW is of great importance for both developed and developing countries. It can be applied with the aim of reducing the total volume of MSW, thus lowering landfilling costs, and if properly treated after careful source separation it can be a source material for substrate used in greenhouses or to amend soils with OM. It is well known that compost maturity is of great importance from the point of view of the compost quality, as well as for cost optimization of the composting facility. Due to its importance and complexity, the search for ideal parameters of composting maturity is ongoing. Advanced chemical, physical and spectroscopic methods of compost maturity have been reviewed in this work. The bulk compost material has been the focus of earlier studies, yet HS and even more so the DOM and its fractions have become the focus of concern in more recent studies, as well as in the present one. It seems that DOM fractions are the most labile ones within the decomposing OM and therefore, the point at which changes in their content and composition occur seems to be a good indicator of maturity. Examination in detail of the DOM and its fractions with advanced chromatographic, spectroscopic and chemical techniques, along with the evaluation of metal binding to these fractions, was the aim of this review. It has been confirmed that decreased DOC and hydrolyzable carbohydrates are characteristic of the OM decomposition process taking place during composting. Hydrolyzable amino acid concentrations fluctuate, and therefore, cannot serve as indicators of maturity.

Key words: municipal solid waste (MSW); dissolved organic matter (DOM); hydrolysable carbohydrates

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1. INTRODUCTION

Compost amendment is the most attractive approach for organic matter (OM) addition to soils for the following reasons: (i) it exhibits a particle size distribution that favors a uniform application in the field; (ii) the C/N ratio is optimal for the support of plant growth; (iii) compost is usually devoid of weed seeds (as opposed to fresh manure); (iv) it is often suppressive to soil-borne diseases (Hoitink et al., 1993); and (v) it is by far richer than manures in humic substances (HS) (Inbar et al., 1990), thereby enhancing plant growth.

Composting of municipal solid waste (MSW) has become popular in the last two decades in many industrial countries, since it constitutes a sensible alternative to commonly used waste management practices such as waste dumping and incineration (Golueke and Diaz, 1996). This is a direct result of the elevated costs of properly managed sanitary landfills and high-temperature incineration, as well as a higher awareness of the advantages of composting, which are, above all, the recycling of nutrients and OM, the decreased air and water pollution compared to other waste management practices, and the improvement of the quality of soils to which compost is added (He et al., 1992; Golueke and Diaz, 1996; Schulz and Römheld, 1997).

On the other hand, the use of MSW compost for agricultural purposes can involve a variety of problems and environmental risks: yield reduction due to immature composts; the loss of greenhouse-active gases; and leaching of nutrients and pollutants (Gallardo-Lara and Nogales, 1987; He et al., 1992; Vogtmann et al., 1993; Helm, 1995). In addition, MSW composts contain many undesirable substances: foreign inert materials like glass, plastics, metals and wood; organic contaminants like polyaromatic hydrocarbons and polychlorinated biphenyls; and heavy metals, some of which may be taken up by plants or microorganisms (Petruzzelli, 1996; Fricke et al., 1996; Schulz and Römheld, 1997).

Effects of compost amendment on plant growth, under growth chamber, greenhouse, and laboratory conditions were discussed in a number of publications (e.g. reviews by Gallardo-Lara and Nogales, 1987; and Chefetz et al., 1996) In general, positive effects on crop yield were reported in a number of crop species. Many of these studies focused on the general beneficial effects of OM addition to soils. Compost addition improved water retention and enhanced soil porosity. Also, the pH buffering capacity of the soil improved, cation exchange capacity (CEC) increased and the level of soil organic matter (SOM) increased. Nitrogen availability in compost-amended soil depends largely on the maturity of the applied compost, yet the effect of compost application on P, S, Ca and Mg availability and uptake seems to be controversial and varies among reports. Potassium and micronutrient availability was found to improve following compost amendment (Gallardo-Lara and Nogales, 1987). Soil amendment with MSW for periods of 4 years or longer improved the physical properties of soils (Giusquianti et al., 1995) based on the same parameters mentioned above. An increase in the HS level, CEC and microbial activity were also observed by these investigators.

The water soluble fraction of natural organic matter (NOM) is the major form of OM in water, and yet, quantitatively, a minor fraction of SOM, or compost. This fraction is usually called dissolved organic matter (DOM) or dissolved organic carbon (DOC). Due to its high capacity in binding micronutrients such as Fe, Zn, Mn and Cu, and/or toxic heavy metals (such as Hg, Cd, Ni, Pb and others) it is of great importance to their bioavailability to plants and microorganisms. In addition, DOM is known to strongly sorb xenobiotics, thereby playing a major role in their transport and reactivity in the environment. The properties of compost derived DOM were shown to provide important information on the transformations occurring in the OM during its decomposition.

An example of the importance of compost derived DOM in the microelement nutrition of higher plants was given in experiments conducted with nutrient solutions (NS) enriched with compost-derived DOM (a 1:10 solid:water extract of compost) (Chen et al., 1994). These investigations showed that growth was significantly enhanced by the DOM, reaching a maximum at a level of 35-55 mg/L. This level was found to be optimal for root and shoot weight, leaf chlorophyll concentration, leaf area, and plant height. A higher concentration of the DOM resulted in growth inhibition when compared to the optimum level.

All metal and xenobiotic binding reactions strongly relate to the chemical nature and reactivity of the DOM. These properties, however, change during composting and biodegradation of the organic materials. Transformations of OM during composting have been studied extensively (Jimenez and Garcia, 1992; Chen and Inbar, 1993; Chiavatta et al., 1993; Chefetz et al., 1996). Most of the published work focused on water insoluble HS. The characterization of DOM seems to have the potential of being an indicator for the transformation and changes in composition occurring during the different stages of composting (Chefetz et al., 1998a, b).

In accordance, the objectives of this study were: (i) to investigate the transformations of DOM originating from composted MSW at different stages of composting as corroborated from the chemical, chromatographic and spectroscopic characterization of its fractions. This report will include fractionation of the DOM and the various fractions will be characterized using chemical, chromatographic, and spectroscopic methods; and (ii) to evaluate compost stabilization based on changes in the composition of the DOM.

2. ORGANIC MATTER COMPLEXES WITH METALS: NATURE, PROPERTIES, AND EFFECTS

A complex of a metal ion and organic molecule arises when water molecules surrounding the metal ion are replaced by other molecules or ions, with the formation of a coordination compound. The organic molecule that combines with the metal ion is commonly referred to as the ligand. Examples of groupings in organic compounds that have unshared pairs of electrons, and that can form coordinate linkages with metal ions are in the order of decreasing affinity of organic groupings for metal ions as follows (Stevenson, 1994):

Table 1. Examples of groupings in organic compounds having unshared pairs of electrons, (after Stevenson, 1994).

- 0 -	>	$-NH_2$	>	-N = N	>	-N	>	$-COO^-$	>	- 0 -	>	C = 0
Enolate		Amine		Azo		ring N		carboxylate		ether		Carbonyl

If the chelating agent forms two bonds with the metal ion, it is called bidentate; similarly, there are tridentate, tetradentate, pentadentate and hexadentate complexes. The formation of more than one bond between the metal and the organic molecule usually results in high stability of the complex. The stability of a metal-chelate or complex is determined by such factors as the number of atoms that form a bond with the metal ion, the number of rings that are formed, the nature and concentrations of the metal ions, and pH. The stability sequence for some complexes of HS with divalent cations is as follows:

$$Cu^{2+} > Ni^{2+} + Co^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+}$$

Detailed reviews of various modeling approaches to binding of metals to HS have been published (Stevenson, 1994) and will not be described here. Stability constants for various metal ions with HS were presented by Chen and Stevenson (1986), but it should be noted that those for Fe^{3+}, which is of great importance to plant growth (Chen et al., 2004) were reported in the literature to a very limited extent and only for very low pH (<3.0).

The formation of metal-organic complexes has the following effects on the micronutrient cycle in soils (Chen and Stevenson, 1986; Stevenson, 1994): (i) Micronutrient cations that would ordinarily precipitate at the pH values found in most soils are maintained in solution through complexation with soluble organics. Many biochemicals synthesized by microorganisms form water-soluble complexes with trace elements. Complexes of trace elements with fulvic acid (FA) are also water soluble. Complexes of HA with trace metals such as Fe are likely to form colloids that will remain in solution or form suspended particles. Either of these conformations will contribute to the mobilization of the metal to the plant root; (ii) Under certain conditions, metal ion concentrations may be reduced to a nontoxic level through complexation with SOM. This is particularly true when the metal-organic complex has low solubility, such as in the case of complexes with humic acids (HA) and other high-molecular-weight components of humus; (iii) Various complexing agents mediate transport of trace elements to plant roots, and, in some cases, to other ecosystems, such as lakes and streams; (iv) Organic substances can enhance the availability of insoluble phosphates through complexation of Fe and Al in acid soils, and Ca in calcareous soils; (v) Chelation plays a major role in the weathering of rocks and minerals. Lichens, for example, enhance the disintegration of rock surfaces to which they are attached through the production of chelating agents.

It was recently demonstrated (Leita et al., 2001; De Nobili et al., 2002; Catalano et. al., 2002) that HS actually interact in solution not only with metal ions, but also with free ligands and complexes of high stability, without causing any ligand exchange. The contribution of HS to the migration of micronutrients and toxic elements might therefore be much more complex than previously considered.

3. METALS IN COMPOST DERIVED DISSOLVED ORGANIC MATTER (DOM): THE MUNICIPAL SOLID WASTE (MSW) CASE STUDY

3.1 Sampling

MSW samples were collected and composted over about 6 months and the properties of the compost were evaluated as described below.

3.1.1 Samples IS1 – Pilot Plant Compost (PPC)

Fresh MSW was collected over a 24 h period from the town of Afula (Israel). The mechanically mixed MSW was sieved (< 5 cm). Compost was prepared using a PPC system (1 m^3 plastic box – 3 replicates) over a period of 187 days. The compost was turned and mixed once a week during the first 100 days of the process, then every 2 weeks. After defined periods twelve sub-samples (days: 6, 12, 19, 25, 33, 47, 62, 77, 105, 126, 161, and 187) were taken and dried at 65 °C for 48 hours, ground and sieved (< 2 mm). These samples were collected during the composting process from each box. A detailed description of the process was given by Chefetz et al. (1998 a).

3.1.2 Samples IS2 – Commercial Compost (CC)

Commercial compost preparations of MSW were collected from the town of Afula. The compost was prepared in windrows, turned every 10 days for 6 weeks, and then every 3 weeks. After defined periods, 5 subsamples (days: 6, 19, 33, 62, and 105) were taken and dried at 65 °C for 48 hours, ground and sieved as above.

Sample designation numbers for both composting series are given in Tables 2 and 3.

3.2 Preparation of Aqueous Extracts

3.2.1 Extract IS1

Ten g of dried material were shaken with 100 mL distilled H_2O for 24 h (1:10 solid:water ratio). After centrifugation (5000 rpm) the samples were filtered (0.45 µm polycarbonate). The liquid samples were used for further characterization (IS1-1 to IS1-12, from t = 6 to 187 days.)

3.2.2 Extract IS2

The dry compost material was ground and the fraction > 2 mm was removed by sieving. Twenty g of solid dry material were extracted with 200 mL deionized water. Ten g of solid dry material were extracted with 100 mL deionized water for the duplicate samples. The samples were shaken for 24 h on a horizontal shaker. Solid-liquid separation was carried out by centrifugation (15 min, 2500 rpm, Heraeus Varifuge 3.2 RS). Finally, the supernatant was filtered through a glass fiber filter and then through a 0.45 µm polycarbonate filter.

3.3 Physico-chemical Properties of the Dissolved Organic Carbon (DOC) and its Fractionation

DOC was determined after the membrane filtration (0.45 µm polycarbonate) using a DOC analyzer (Shimadzu, TOC-5000). The relative standard deviation was ± 2%. Preparative DOM fractionation was performed according to Leenheer (1981) with some modifications. Details of the procedure can be found in Chefetz et al. (1998a). The following fractions were obtained:

Hydrophobic fraction - sorbed to XAD-8 (Ho):

- Ho Base (HoB) - desorbed with 0.1 M HCl
- Ho Acid (HoA) - desorbed with 0.1 M NaOH
- Ho Neutrals (HoN) - desorbed with MeOH

Hydrophilic fraction - not sorbed to XAD-8 (Hi) (obtained after sample elution through XAD-8 column):

- Hi Base (HiB) - sorbed to H^+ cation exchange resin, desorbed with 0.1 M NH_4OH
- Hi Acid (HiA) - sorbed to anion exchange resin, desorbed with 1.0 M NaOH
- Hi Neutrals (HiN) - remaining in deionized-water solution after all column separation.

3.4 Spectral Absorbance (Abs-254)

The spectral absorbance at $\lambda = 254$ nm was measured at pH = 7 using a spectrophotometer (Lambda 5, Perkin Elmer) and a 1 cm quartz cell. Double distilled water was used as a reference. The samples were diluted to a DOC concentration in the range of 4 to 5 mg/L.

3.5 Hydrolyzable Amino Acids (hAA)

For the hydrolysis, 2 mL 6 M HCl were added to 2 mL of the liquid sample. The hydrolysis was carried out at 110 °C for 16 hours. The resulting solution was evaporated to dryness and then dissolved in water. The evaporation step was carried out for three consecutive times. The final residue was dissolved in 0.5 mL water. After a filtration step (0.45 µm cellulose-acetate), the sample was subjected to amino acid analysis, using HPLC-separation. The released hAA were determined using a fluorescence detector (Hewlett Packard 1046 A) after a precolumn derivatization (o-phthalaldehyde, 9-fluorenylmethyl-chloroformate) and HPLC-separation (Hewlett Packard 1090 A). Detailed descriptions of the methods were given by Jahnel et al. (1991, 1998a, 1998b). The HPLC separation was conducted in triplicate and the relative standard deviation for the different hAAs was < 0.3 µM/L.

3.6 Characterization of the Aqueous Extracts (IS1 and IS2)

The pH values of the aqueous extracts of the compost samples exhibited a slight increase with composting time. In general, the DOC concentration in the water extracts decreased with composting time for both pilot plant compost (PPC) and the commercial composting (CC) facilities (Table 2 and 3).

Table 2. Dissolved organic carbon (DOC) and hydrolyzable amino acid (hAA) concentrations, pH, and normalized UV Absorbance (Abs-254 / DOC) of the DOC fraction of MSW compost (pilot plant composting scale - PPC), as a function of composting time.

Sample designation no.	Days of composting	DOC mg/L	pH at 22 °C	Abs-254 / DOC[1)] L/mg·m	C-hAA / DOC µg/mg
IS1-1	6	716	7.4	1.35	68
IS1-2	12	1988	7.7	1.34	80
IS1-3	19	1228	7.9	1.34	88
IS1-4	25	812	8.2	1.57	77
IS1-5	33	890	8.0	1.76	70
IS1-6	47	752	8.1	2.01	82
IS1-7	62	725	8.1	2.38	97
IS1-8	77	766	8.1	2.08	107
IS1-9	105	463	8.3	2.37	86
IS1-10	126	444	8.2	2.43	75
IS1-11	161	357	8.2	n.d.	106
IS1-12	187	417	8.2	2.70	n.d.

[1)] at pH = 7.0; n.d.: not determined

Table 3. Dissolved organic carbon (DOC) concentration, pH, and normalized UV Absorbance (Abs-254 / DOC) of the DOC fraction of MSW compost (commercial scale composting – CC), as a function of composting time.

Sample designation no.	Days of Composting	DOC mg/L	pH at 22 °C	Abs-254 / DOC L/mg·m
IS2-1	6	720	7.8	1.30
IS2-2	19	460	7.7	2.06
IS2-3	33	225	7.9	2.84
IS2-4	62	290	7.9	2.71
IS2-5	105	325	7.8	2.70

[1)] at pH = 7.0; n.d.: not determined

The general trends were similar for both the PPC and CC. During the first phase of composting of up to 12 days of the PPC composting cycle, a sharp increase from an initial DOC concentration of about 715 mg/L up to about 2000 mg/L was observed in the pilot plant scale composting only (Table 2). This increase, which resulted probably from enhanced microbial activity, possibly anaerobic, did not occur in the CC facility composts (Table 3). However, immediately after this initial stage a continuous decline in DOC took place. Towards the end of the process, the DOC reached a plateau (105 days and on) at a concentration of about 420 to 450 mg/L. Similar trends were obtained for the commercial compost (IS2). It appears therefore, that the DOC is, as suggested earlier by Chen and co-workers (Zmora-Nahum et al., 2005), is a good indicator of compost maturity. The DOC concentrations of the commercial compost samples were lower at all stages of composting and the plateau was achieved much earlier (after 33 days), probably, due to higher composting efficiency (Table 3).

The composition of the dissolved organic substances changed with composting time leading to higher spectral absorbance per mg C, thus suggesting the formation of, or selection for, aromatic structures, or of carboxylic and carbonylic electron systems and their conjugates as reported by Inbar et al. (1989) and Chefetz et al. (1998a, b). Even during the stabilization phase from day 105 and on, changes were obvious. Similar observations were found for the CC samples (Table 3).

The specific analysis of hydrolyzable amino acids (hAA) exhibited values ranging from 68 to 106 (n=85) μg/mg (C-hAA / DOC). No clear trend was identified for the normalized concentration of the hAA thus suggesting that no specific degradation or enrichment of this fraction took place. It should be noted however, that in absolute terms a significant decrease was observed for the total level of hAA present in the samples (e.g. 108 mg C in hAA in the sample IS1-3, 19 days; and only 45 mg C in hAA in the sample IS1-11, 161 days). Obviously, there would be no advantage of using the concentration of hAA as an indicator for compost maturity, since the DOC is an easier parameter to measure and it also varies on a much broader scale with composting time (Chou, 2003; Zmora-Nahum et al., 2005).

3.7 Characterization by Chromatography - LC-UV/DOC and Dissolved Nitrogen (DN)

The molecular weight distribution within the DOM fraction was determined using LC-UV/DOC according to Huber and Frimmel (1991, 1994) (column: length = 30 cm, radius = 0.8 cm; eluent: 0.025 M phosphate buffer at pH = 6.8; flow rate: One mL/min; injection volume: 0.5 mL) using a TSK-HW-40(S) gel and on line DOC-detection (DOC Analyzer-Gräntzel). The schematic experimental set up is shown in Figure 1 (upper part).

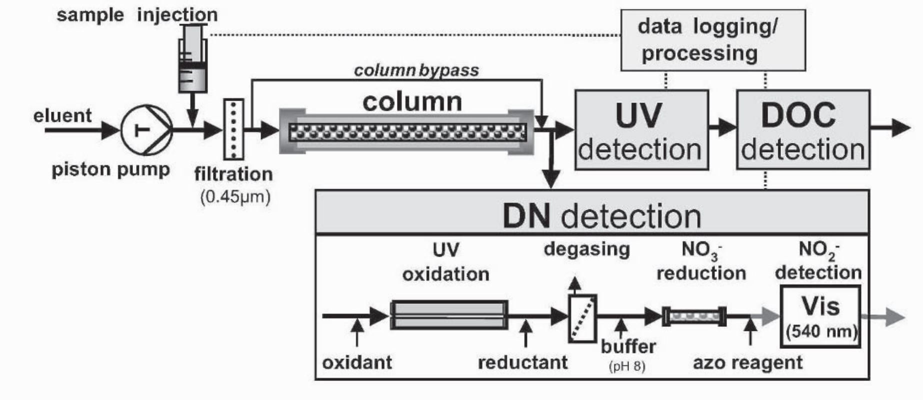

Figure 1. The LC-UV/DOC/DN system used for the characterization of directly injected dissolved organic matter.

As already shown in the literature, size exclusion chromatography (SEC) can be used for the characterization of DOM (Swift, 1985; DeNobili and Chen, 1999). In SEC columns the OM is fractionated according to size, charge and hydrophobicity. The combination of chromatographic separation with online detection of the UV absorption (at $\lambda = 254$ nm) and the determination of DOC facilitated further characterization of DOM thereby leading to an enhanced understanding of the humification process (Huber and Frimmel, 1994; Wong et al., 2002; Perminova et al., 2003).

The total DOC concentration ($< 0.45\mu m$) of the IS1 samples was determined by bypassing the chromatographic system. Only 9 to 15 per cent of the total DOC was not eluted by the chromatographic method applied. Since no trends of change were observed between samples of different composting age, it can be concluded that the irreversibly absorbed DOC fraction, which exhibited a high hydrophobic character, was present at each of the composting stages. Typical chromatograms of molecular size fractionation by the SEC are shown in Figures 2 and 3. We will not discuss the DN data in this paper, since they are preliminary and require further confirmation.

In general the chromatograms obtained by DOC detection and those determined by UV absorption exhibited the same shape (Figure 3 top and bottom). Based on earlier research work, the fractions in the chromatograms can be assigned to different substances. The fraction eluted between the retention times $25 < t_R < 35$ min. was attributed to high molecular weight material (highly condensed and polymerized material) as evident from the analyses of natural HS. Between 50 to 60 per cent of the DOC of the aqueous extracts was attributed to this fraction (fraction 1). The relative amount was about the same for all samples.

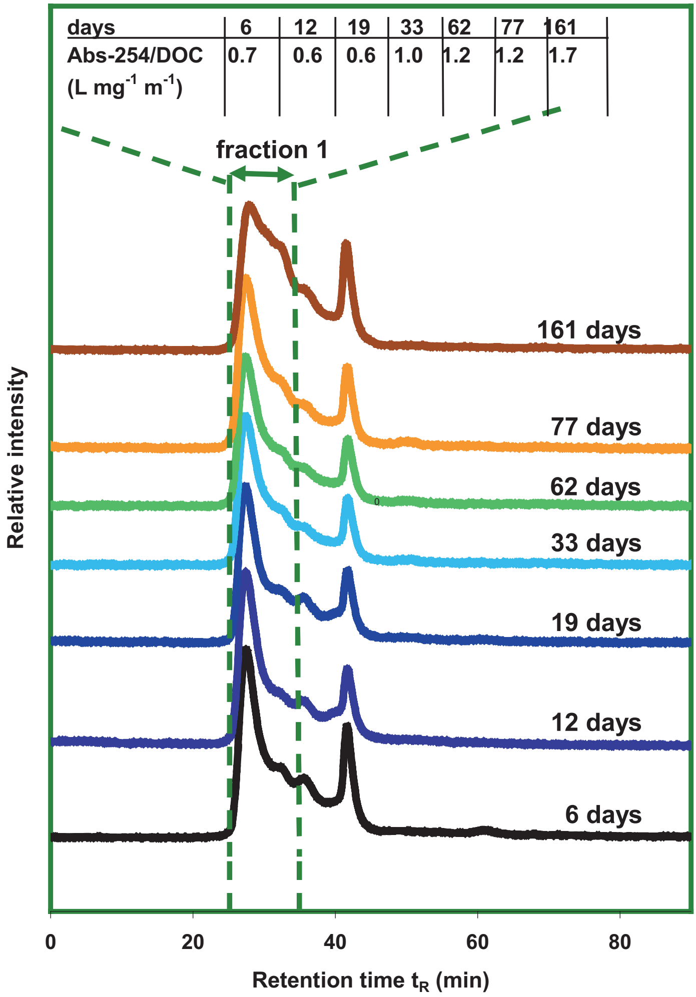

Figure 2. Gel chromatographic separation of the water soluble fractions of DOC extracted from MSW compost at several stages (days) of the composting process. The DOC concentration in each sample was the same.

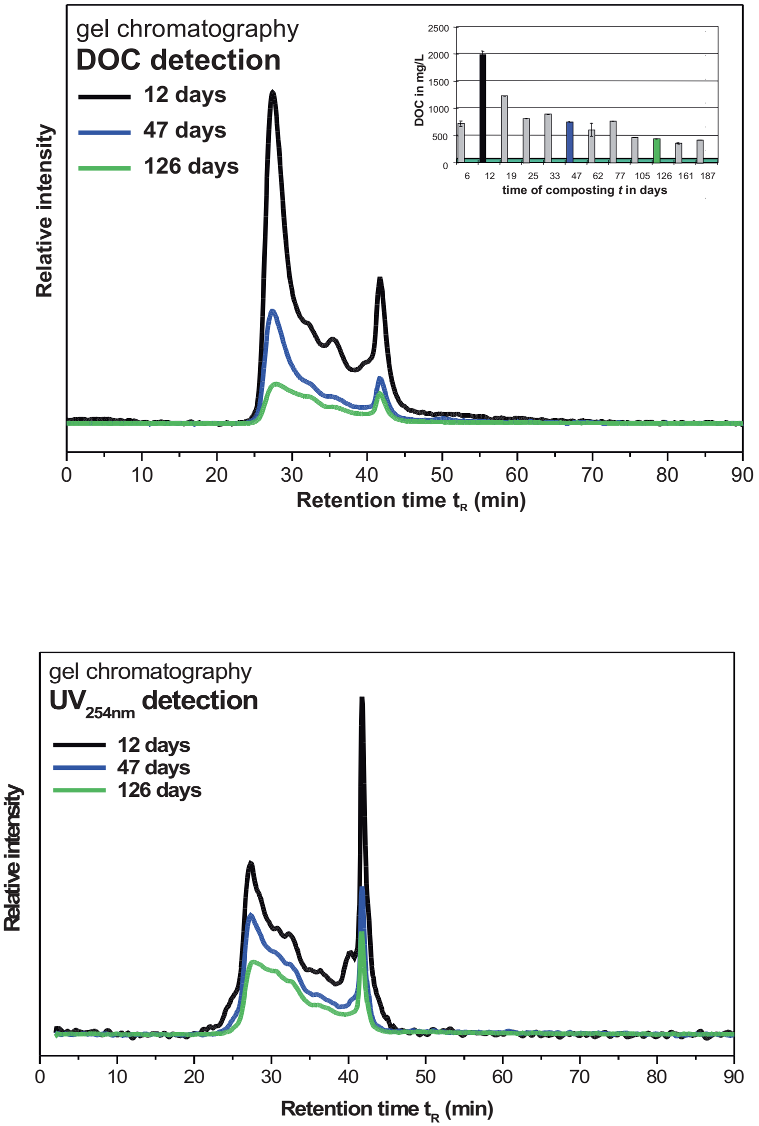

Figure 3. LC-UV/DOC chromatograms for selected IS1-samples.

The average UV absorbance of each fraction was calculated to examine the spectral absorbance at λ=254 nm relative to the DOC (Abs-254/DOC), as was carried out earlier by direct measurements employed on the DOM samples. The Abs-254/DOC values of fraction 1 increase with time of composting. After 19 days of composting this ratio increased exponentially due to an increase in the degree of humification. The results are in accordance with a report by Chefetz et al. (1998a, b) showing an increase in the amount of hydrophobic fractions as result of the fractionation of the aqueous extracts of the MSW material after applying XAD8 and amberlite resins separations (according to Leenheer, 1981).

4. DERIVING METAL BINDING SITE STRENGTH AND CAPACITY FROM EXPERIMENTAL DATA

A straightforward approach to determine the complexing capacity (CC) is to plot M_F/L_C (M_F: free metal concentration in solution; L_C: concentration of ligand) against M_T/L_C (Figure 4). Due to metal complexation by the ligand, the line of data points will assume a slope = 1 when $M_T/L_C >> CC$. M_F will accumulate in the solution only after the CC of the ligand has been approached. Hence, a linear regression of all data points with $M_F > 0$ using the least-squares method approximates the CC as the *x*-axis intercept with the corresponding standard error.

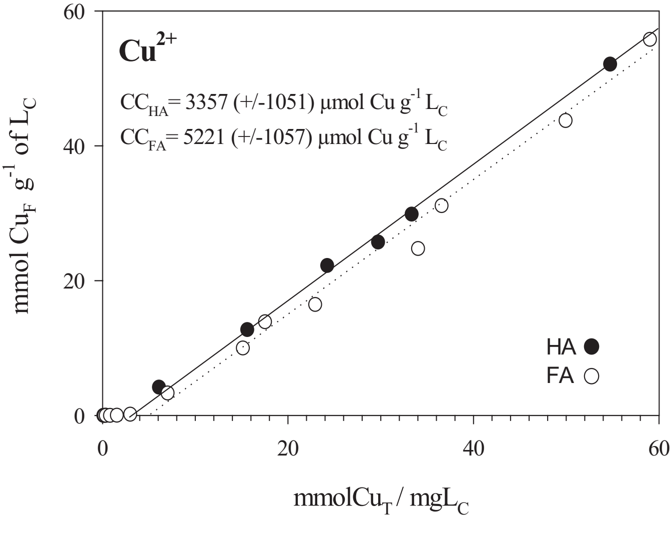

Figure 4. Deriving a complexing capacity from titration data: MF/LC is plotted against MT/LC followed by linear regression of all data with a free ion concentration > 0. In the example, humic acid (HA) and fulvic acid (FA) from compost were titrated with a Cu-perchlorate solution. CC: complexing capacity.

To characterize the strength of bonding, stability constants are calculated. For a given ligand with discrete homogeneous binding sites for a metal ion, the titration data can be plotted in Hill or Scatchard plots, which yield the desired thermodynamic data as the slope and y-intercept of a linear fit (Stevenson, 1994). For HS, however, the Scatchard plot is always curvilinear, as a result of the range of binding sites (BS) with slightly different binding energies rather than a discrete number of sites of defined binding strength. ML_2 (metal binding to functional groups on two ligands) complex formation has also been suggested as contributor to the curvilinear nature (Stevenson, 1994).

Since the two-component Scatchard plot yielded unsatisfying results for an interpretation of the curvilinear nature and the subsequent calculation of stability constants (see section above), different models have been developed to better quantify the binding strength and interpret the experimental data (Dzombak et al., 1986; Fish et al., 1986; Stevenson, 1994). In general, researchers have moved away from using a discrete site approach to using models providing for a wide range of binding site strengths on the molecule, the so-called continuous models. Continuous models are employed to address the fact that metal binding by humic substances apparently cannot be explained by a limited number of binding sites with discrete binding strengths. One such continuous distribution model is based on a normal distribution of binding site strengths (Perdue and Lytle, 1983; Dzombak et al., 1986; Fish et al., 1986; Manunza et al., 1992; Stevenson, 1994; Manunza et al., 1995). To account for the fact that chemically different groups on the macromolecule are relevant for binding, Manunza et al. (1995) have proposed the use of a bimodal approach for the distribution model. Such a model hypothesizes that two main types of binding groups, namely carboxyl and phenol groups, account for most of the complexation.

A successful approach to derive meaningful binding strength data calculates incremental stability constants from successive slope values in the Scatchard plot, which are subsequently plotted according to their frequency on the molecule using the Gaussian normal distribution (Stevenson and Chen, 1991; Stevenson et al., 1993; Logan et al., 1997; Kaschl et al., 2002). In this way, the strongest binding groups, the full range of binding site strengths on the molecule and the most abundant binding site strengths on the molecule may all be quantified. This methodology is described below.

It is assumed that the HS molecule (L) is the central group to which each metal ion (M) is bound to a single reactive site (formation of LM, LM_2, LM_n complexes). The slope of the Scatchard plot (ν/M_F plotted against ν with $\nu = M_B/L_C$; M_F = free 'ionic' metal, M_B = bound metal, L_C = ligand concentration as dissolved organic carbon (DOC)) is interpreted as the stability constant K_0 between the ligand and M. For HS it forms a curve, for which successive (incremental) slope values may be calculated from neighboring data points in the Scatchard plot (slope = $(\nu/M_F) / \nu$); Figure 5A). These slope values are conditional stability constants K_i at a certain value of ν', which in turn lies equidistant between the original neighboring data points (used to calculate the incremental slope) on the ν-axis of the Scatchard plot. This approach is superior to fitting a polynomial equation to the Scatchard plot data and calculating the derivative, since the inherent error of polynomial fitting would be multiplied when the derivative is formed (Kaschl et al., 2002).

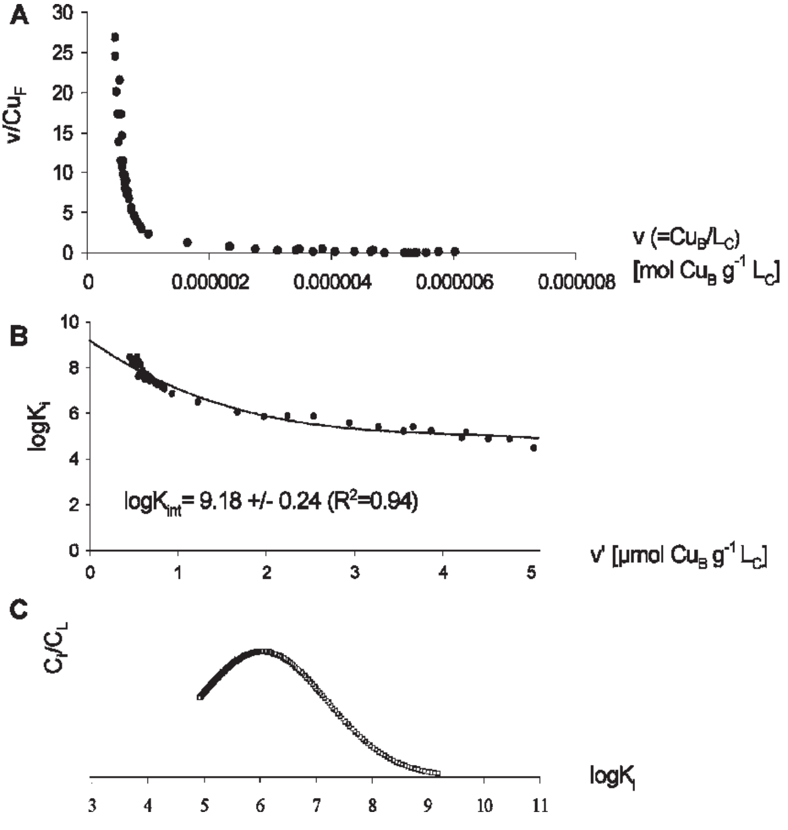

Figure 5. Derivation of logKint (pKint) and a continuous distribution of binding sites from the Scatchard plot. A: Scatchard plot for the titration data of compost fulvic acid and Cu (two replicates), B: Incremental stability constants logKi calculated for each interval v' and polynomial fit, C: relative abundance of binding sites vs. logKi obtained with a continuous model based on a normal distribution. logKi: incremental binding site strengths, Ci/CL: mole fraction of binding sites at logKi, Lc: ligand concentration as DOC.

A plot of $\log K_i$ against ν' displays the range of binding site strengths observed. Fitting a 3rd-degree polynomial to the data of this K_{int} vs. v' plot and extrapolating towards $\nu' = 0$ gives the intrinsic constant K_{int} for binding at the strongest sites (Stevenson and Chen, 1991; Stevenson et al., 1993) (Figure 5B). These sites may be of particular interest since they would be the first to be occupied and therefore of special relevance in a natural environment (Stevenson, 1994). Studies using this approach have shown that the $\log K_{int}$ for humic macromolecules is several orders of magnitude higher than logK measured by other approaches (Stevenson et al., 1993; Stevenson and Chen, 1991; Kaschl et al., 2000; Kaschl et al., 2002). For Cu, $\log K_{int}$ values ranged from 7 to 9 for HA and FA (Stevenson and Chen, 1991; Kaschl et al., 2000). However, these strong binding sites are element-unspecific, and therefore are likely to be occupied by cations occurring in elevated concentrations in the natural environment (e.g. alkali-earth cations).

To model the distribution of binding site strengths on the HS molecule, values for incremental binding strengths $\log K_i$ are calculated at equal intervals of $\underset{\cdot}{\nu}$ from the polynomial fit to the data in the ($\log K_i$ vs. v')-plot (Figure 5B)(Stevenson and Chen, 1991). Assuming a normal distribution, the relative abundance of binding sites within the incremental interval δpK_i can be calculated with the Gaussian distribution function (Stevenson and Chen, 1991). The curve obtained shows the range of binding sites on the humic molecule in addition to their frequency (relative abundance) (Figure 5C). A larger range of binding sites for a specific HS molecule may indicate a greater variety of binding site types present (Kaschl et al., 2000; Kaschl et al., 2002). With the help of this continuous distribution model, humified organic ligands in DOM were shown to have a greater range of binding site strengths as opposed to less humified and non-humic ligands such as polysaccharides or proteins (Kaschl et al., 2000; Kaschl et al., 2002).

In addition to the range of binding site strengths, the $\log K_{int}$ (stability constant for the strongest groups) appears in the continuous model as the extreme value on the right hand side of each distribution curve where C_i/C_L approaches zero. Consequently it is obvious from Figure 5C that functional groups with binding site strengths close to $\log K_{int}$ represent only a minute percentage of total metal-binding groups on the ligands. Therefore, the quantification of the $\log K_f$ may be more relevant for a characterization of HS binding strengths and for use in fate modeling. This parameter refers to the most abundant binding site strength on the molecule and constitutes the $\log K_i$ value at the peak of the distribution curve. In a multi-species environment such as the soil solution, this parameter could be helpful to characterize the most likely binding strength of metal-humic associations. Its value ranged from 5 to 6 for Cu at pH = 5 and about 5 for Cd at pH = 7 in the studies using this modeling approach (Stevenson and Chen, 1991; Kaschl et al., 2000; Kaschl et al., 2002).

The distribution curves obtained for HS were skewed, indicating a deviation from the Gaussian distribution (Stevenson and Chen, 1991; Kaschl et al., 2000; Kaschl et al., 2002). In addition, they were generally flatter than those obtained for other organic ligands in the soil solution, presumably indicating a higher number of differing reactive sites (Kaschl et al., 2000; Kaschl et al., 2002). Other recent modeling approaches for trace metal binding by HS have focused on thermodynamic considerations (Kim and Czerwinski, 1996; Bryan et al., 1998; Bryan et al., 2000), a random structural approach to identify the likely metal binding sites (Bryan et al., 1997), kinetic aspects (Yu et al., 1996), discrete binding sites (Bolton et al., 1996; Woolard and Linder, 1999) and a combination of electrostatic considerations and site binding models (Benedetti et al., 1996; Kinniburgh et al., 1999; Gustafsson, 2001; Koopal et al., 2001; van den Hoop et al., 2002).

5. EFFECTS OF DOM AND ITS FRACTIONS ON PLANT GROWTH

DOM before fractionation, as well as HoN and HoA were obtained by our group from a mature MSW compost (Amichai, 2001). The latter fractions were selected because their levels increase with degree of maturity and correlate with plant growth response. The HoN level in mature compost is higher than in soils due to the enhanced humification in composts. The HoA resembles soil HAs (Guggenberget and Zech, 1994; Chefetz et al., 1998a, b).

The addition of either DOM, HoN or HoA to NS enhanced their ability to maintain Fe^{3+} in solution (or in a colloidal form) at pH 6 or 7.3 far beyond the levels observed in the control solutions. The option of the formation of colloidal or finely suspended conformations of organo-Fe complexes has also been considered, although further studies on these forms are required. Sticher (1997) and Dolfing et al. (1999) investigated the adsorption of Fe hydroxides on surfaces of organic macromolecules. According to their findings, further crystallization is inhibited by the surface adsorption. This hindrance is likely to enhance the availability of the Fe to plants. The molecular sizes of the colloids formed in the NS were studied by our group using size exclusion chromatography (SEC), and will be reported elsewhere.

The apparent stability constants (K_{app}) were determined by our group for Fe^{3+} with the DOM and its two fractions using a colored ligand exchange method. The K_{app} values at pH 5 and ionic strength (μ) of 0.1 M for the

DOM, HoA and HoN were 6.88, 7.91, and 6.76, respectively. The DOM and its two fractions were further tested to evaluate their potential contribution and note in the Fe nutrition of plants. A distinctive positive response to the Fe addition will substantiate the hypothesis that the growth stimulation of plants by HS is basically an Fe nutrition (and possibly additional micronutrients such as Zn) dependent mechanism.

In earlier experiments, peanuts (*Arachis hypogea*) were shown to provide a good bioassay system for the Fe nutrition status of plants (Barak and Chen, 1982), and therefore they were used as test plants for the efficacy of the DOM, HoN and HoA as Fe carriers to plants.

Plant growth trials were conducted in NS buffered to pH 7.3 by excessive $CaCO_3$, thus providing a simulation of calcareous soil conditions. Chlorophyll concentrations of the leaves which serves as an indicator for the Fe nutrition status of the plants (green plants = high chlorophyll concentration = sufficient Fe supply; yellow plants = low chlorophyll concentration = deficient Fe supply), were significantly higher in plants grown in NS solution containing 50 mg/L of either DOM, HoN or HoA, compared to plants supplied with a mineral form of Fe only. Plants supplied with the FeHS complexes were as healthy and green as those supplied with FeEDDHA, which is the best (for dicotyledonous plants such as peanuts) Fe-chelate known to date. Plants supplied with $FeSO_4$ only, turned yellow during the growth period, and at the end of this period they did not differ from control plants (grown without any Fe supply). Similar results were obtained by Chen et al. (2001), who tested effects of HA or FA on both monocots and dicots. These results are in accordance with a series of studies by our and other groups showing the significance of organo-Fe complexes to plant nutrition (Chen and Barak, 1982; Chen and Stevenson, 1986; Chen, 1996). Recent studies by Pinton et al. (1999) also showed the correction of Fe deficiency in cucumber plants (*Cucumis sativus*) using Fe humates. The experiments summarized in this section clearly show the importance of HS to the supply of Fe to plants.

6. CONCLUSIONS

Different experimental and modeling approaches have been used to describe metal binding processes and an extensive amount of literature is now available on the topic. The functional groups responsible for the binding of metals have been mostly identified, even though the exact local geometry and structure of the ligand and metal complex is still under investigation. The use of X-ray spectroscopy promises to provide new insight on this aspect in the near future. The binding process itself is strongly affected by abiotic parameters such as pH and ionic composition of the soil solution, with important repercussions for immobilization processes.

To predict and model the effect of HS on metal solubility in the field, element-specific thermodynamic parameters describing binding strength and the total binding capacity of HS molecules have been obtained in numerous laboratory studies using several valid methodological approaches. These studies have to a large degree substantiated the Irving-Williams stability series, with the strongest complexation found in the case of Hg, Pb and Cu. The quantification of the binding process, however, is still being improved and investigated. The early approaches, used in the 1970s, to determine stability constants from experimental data such as the two-point Scatchard plot have seriously underestimated the strength of binding. In these studies, the highly soluble fraction of HS, composing soil or water DOM, have been overlooked. They were shown in recent studies conducted by our group (partially summarized in this article) to strongly bind various metals while maintaining high solubility and leachability. These studies have shown that HS binding sites on the same molecule vary in binding strength over a range comprising several orders of magnitude. Hence continuous distribution models appear to be most promising to more accurately describe the ability of HS to retain trace metals. A thorough understanding of the complexation process will help to model the occurring HS-trace metal interactions in the field and increase the chances of HS sorption processes to be used as immobilizing agents within enhanced natural attenuation approaches to contaminated site cleanup.

In the soil environment, HS are the most important organic ligands for trace metals, forming a myriad of both soluble and insoluble complexes. Micronutrients such as Zn and Fe may be held in solution bound to HS and thus remain available to biota, just as much as pollutants may be sorbed and immobilized by HS that bind tightly to the soil matrix. In order to make better use of HS as immobilizing agents for remediation purposes, it is absolutely necessary to develop a good understanding of the underlying processes of HS-trace elements interactions. Even more so since, as demonstrated by their role in plant nutrition, binding to HS does not necessarily result in a containment of trace metals, but may also serve the purpose of increasing mobility and bioavailability of trace elements.

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THE EFFECT OF ORGANIC MATTER FROM BROWN COAL ON BIOAVAILABILITY OF HEAVY METALS IN CONTAMINATED SOILS

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Abstract: Organic matter is able to bind heavy metals. Enrichment of soil with organic matter could reduce the content of bioavailable metal species as a result of complexation of free ions of heavy metals. This process could be used for remediation to protect plants against metal pollution. The experiments were carried out on Haplic Luvisols in field pots sunk into the ground. A brown coal preparation (the "Rekulter") was used as an amendment of soils artificially contaminated with Cd, Pb and Zn. A winter rye was used as the test plant. The total content of heavy metals and concentration of their bioavailable species in the soil were determined using 2M HNO_3 and EDTA extraction, respectively. The content of bioavailable (soluble in EDTA) zinc, lead, cadmium in soil depended on the application rate of the "Rekulter" and decreased along with an increase in the amendment dosage. The highest efficiency of the "Rekulter" in preventing uptake of zinc, lead and cadmium by plants was observed for the largest dosage of this preparation. The bioaccumulation (BI) indexes of Cd, Pb, Zn indicate availability of Cd, Pb and Zn to plants. The BI decreased along with an increase in the brown coal amendment dosage.

Key words: heavy metals; organic carbon; contaminated soil; brown coal; bioavailability

1. INTRODUCTION

Contamination of soil by heavy metals is of major concern because of their toxicity. Sources of heavy metals are traffic dust emitted into the atmosphere, residues of sewage sludge and other waste materials discharged into soil, mineral, mainly phosphorus, fertilizers and some plant protection

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chemicals (Fergusson, 1990). Elevated levels of heavy metals in soils may lead to their uptake by plants, which depends not only on heavy metal contents in soils but is also determined by soil pH value, organic matter and clay contents, and influenced by the fertilization (Fytianos et al., 2001). The fluctuation of mentioned parameters cannot change the total amount of heavy metals in soil but can significantly affect their bioavailability (Ge et al., 2000; Römkens et al., 1999). In recent studies, organic matter has been implicated for alleviating bioavailability of heavy metals in soils. Some researches showed that amendment of contaminated soils with organic matter reduced bioavailability of heavy metals (Kham et al., 2000).

Soil organic matter has been of particular interest in studies of heavy metal sorption by soils, because of tendency of transition metal cations to form stable complexes with organic ligands (Elliott et al., 1996). Organic matter is known to form strong complexes with heavy metals (Krogstad, 1983). The content of organic matter affects speciation of heavy metals in soil (Lo et al., 1992). High organic matter content was reported to decrease concentrations of Cd and Ni in soil solution (Arnesen and Singh, 1999).

Application of brown coal preparations as a source of organic matter is a well-known practice to improve soil properties (Kwiatkowska et al., 2005). Organic matter from brown coal undergoes slow mineralisation and that is why it is very good material as a source of organic matter in soil especially, light soil. This is very important because a high content of organic matter in contaminated soil is one of the ways to exclude heavy metals from the trophic chain.

The objective of this study was to prove the feasibility of using brown coal-derived preparations for reducing bioavailability of cadmium, lead and zinc to higher plants. The use of brown coal in agricultural production, protection of soil ecosystems and land reclamation has been described previously (Maciejewska and Kwiatkowska, 1997, 2000), but BI were not calculated for plants.

2. MATERIALS AND METHODS

The experiments were carried out in the field in stoneware pots sank into the ground during 1999-2000. The soil used was Haplic Luvisols formed from loamy sand. The soil was artificially contaminated with target heavy metals by mixing it with solutions of $Cd(NO_3)_2$, $Pb(CH_3COO)_2$ and $ZnSO_4$. The pots were then filled with 56.4 kg of either pure or contaminated soil amended with a brown coal preparation “Rekulter” containing 85% of brown coal. The application rate of "Rekulter" was 180, 360 and 720 g per pot, which is equivalent to 5, 10, 20 tons of organic carbon per ha.

In 1999 and 2000, a winter rye (Se*cale cereale*) was grown on the prepared soils and harvested in green forage. After harvest, the soil samples were collected from each pot, air-dried, mixed, sieved (1-mm), and analyzed.

Total organic carbon was determined using TOC-analyzer (Shimadzu 5000). Soil pH was determined potentiometrically in 1M KCl using a 1:2.5 (w:v) soil/solution ratio. Soil samples were extracted with 2M HNO_3 (1:10, w/v) (Andersson, 1975) to determine the total contents of Cd, Pb and Zn. Bioavailable forms of heavy metals were determined using extraction with a chelating mixture composed of 0.017M $EDTAH_4$, 0.01135M $Ca(CH_3COO)_2$ 2 H_2O, 0.019M $C_3H_4(OH)(COOH)_3 \cdot H_2O$, and NH_3 at pH 7.3 (1:4, w/v) (Nowosielski, 1974).

Plants were collected, washed, weighted, cut into pieces, and dried at 60°C until they reached constant weight. Then, 1.0- to 2.0-g subsamples were ground in a stainless steel mill, and mineralised in the liquid mixture of concentrated acids (HNO_3 to $HClO_4$, volumetric ratio of 4:1). Content of lead, zinc and cadmium in the soil and plant samples were determined using inductively coupled plasma-optical emission spectroscopy (ICP-AES) Perkin-Elmer 3000DV. The analyses were carried out in triplicate. For all data processing, a standard statistical software package, Statgraphics 4.1, was applied. One-way ANOVA analyses were undertaken to establish significant differences between treatments.

The bio-accumulation index (BI) was calculated as a ratio of a heavy metal content in a plant to its total content in a soil. BI was used to evaluate mobility of Cd, Pb and Zn in the soil and their availability to plants.

3. RESULTS AND DISCUSSION

The conducted experiments have shown that amendment of soil with the brown coal preparation studied changes significantly physicochemical properties of the soil (Table 1). The highest increase in pH (pH_{KCl}=6.30) was found in the soils with the highest dose (720 g per pot) of the "Rekulter" (R) per pot. Importance of soil pH value for for availability of heavy metals to plants was numerously reported (Fytianos et al., 2001; Elliot et al., 1986). Solubility of heavy metals is highly pH dependent and, as a rule, it increases along with a decrease in soil pH. Heavy metal sorption mechanisms in soils are influenced by soil pH, and, hence, soil sorption capacity will be greatly affected by any changes in pH. This means that the availability of heavy metals for plants in our experiment was likely to be the highest in the treatment without 'Rekulter' (pH = 5.05) and the least where the highest rate of this preparation was applied (pH = 6.30).

Table 1. Soil pH in 1M KCl and content of organic carbon in soil in the soils treated with different application rates of the "Rekulter", mean values.

Dose of Rekulter [g per pot]	pH_{KCl}	Organic carbon [g/kg of soil]
0	5.05	6.34
180	5.87	8.97
360	6.05	10.99
720	6.30	14.10
LSD α=0.05	0.086	1.03

LSD α=0.05 – least significant difference, P=0.05

The smallest dose of 'Rekulter' (180 g per pot) introduced into the soil about 9.0 [g/kg of soil] of organic carbon (Table 1). Soil used in this experiment was light, and mineralisation of organic matter originating from organic fertilizer, for example farmyard manure, is rather fast. Finding quite a big content of organic matter in this experiment confirms the results obtained by Maciejewska and Kwiatkowska (2000), Kwiatkowska et al. (2004).

The content of cadmium, lead and zinc in 2 M HNO_3 extract from soil did not correlate with the application rate of the 'Rekulter', but there was a significant decrease in the content of these heavy metals in all treated pots as compared to non treated soil (Table 2).

Table 2. The content of Cd, Pb, and Zn species soluble in 2 M HNO_3 and in the chelating mixture (EDTA) [mg/kg of soil] in the soils treated with different application rates of the "Rekulter", mean values.

Dose of Rekulter [g per pot]	Cd- soluble in 2M HNO_3	Cd- soluble in EDTA	Pb- soluble in 2M HNO_3	Pb- soluble in EDTA	Zn- soluble in 2M HNO_3	Zn- soluble in EDTA
0	1.910	1.324	98.4	48.00	119.9	73.0
180	1.630	0.612	95.8	42.00	113.7	70.3
360	1.595	0.592	95.7	41.30	112.5	69.2
720	1.600	0.585	95.5	32.04	114.0	52.4
LSD α =0.05	0.096	0.008	1.78	0.72	2.41	0.79

LSD α=0.05 – least significant difference, P=0.05

The content of Cd soluble in EDTA was approximately 70% of that soluble in 2M HNO_3 extract in the pots, which were not amended with the 'Rekulter', and about 37% in the pots amended with the brown coal preparation. The corresponding values for Pb and Zn were 49% and 61%, respectively, for the pots with the smallest application rate of the "Rekulter" and 33% and 45%, respectively, for the pots amended with the highest dose of the "Rekulter". These results show that the brown coal amendment was very efficient in immobilizing Zn, Pb, and Cd in the soil studied. The efficiency of metal immobilization was the highest for cadmium and much less - for Zn and Pb. The poorest metal sorption was obtained in the untreated soil. Karczewska et al. (1996) focused on using the brown coal for remediation and reclamation areas polluted with heavy metals, especially, with Cu and Pb. They reported that not only adsorption, but also complexation and precipitation were involved in immobilization of heavy metals in the presence of brown coal amendments.

The contents of Cd, Pb, Zn determined in rye *(Secale cereale)* (roots, stalk and ear) grown on the contaminated soils are given in Tables 3 - 5.

Table 3. Contents of cadmium in rye dry mass (g/kg d.m.).

Dose of Rekulter [g per pot]	Cadmium		
	roots	Stalk	Ear
0	0.48	0.83	0.15
180	0.48	0.23	0.06
360	0.47	0.25	0.06
720	0.20	0.22	0.03
LSD α=0.05	0.017	0.035	0.008

LSD α=0.05 – least significant difference, P=0.05

Table 4. Contents of lead in rye dry mass (g/kg d.m.).

Dose of Rekulter [g per pot]	Lead		
	roots	stalk	ear
0	6.87	2.87	3.34
180	4.73	2.67	2.18
360	5.10	2.54	1.77
720	2.25	2.52	1.57
LSD α=0.05	0.21	0.13	0.11

LSD α=0.05 – least significant difference, P=0.05

Table 5. Contents of zinc in rye dry mass (g/kg d.m.).

Dose of Rekulter [g per pot]	Zinc		
	roots	stalk	ear
0	91.33	311.0	129.0
180	89.73	94.65	71.40
360	67.93	97.5	68.33
720	43.37	64.77	63.5
LSD α=0.05	1.11	1.77	0.87

LSD α=0.05 – least significant difference, P=0.05

Application of the "Rekulter" significantly influenced uptake of Cd, Pb and Zn by the tested plants. Metal concentration in the plants was proportional to metal concentration available for the plant in the soil. It was found that high metal concentration in 2M HNO_3extract did not correlate with its concentration in the EDTA-mixture. The comparative analysis of Cd, Zn, and Pb sorption by and release from the soils treated with different doses of the brown coal organic matter is given below.

Cadmium. The highest decrease of Cd content was found in the pots with the largest application rate of the "Rekulter" (720g per pot) and accounted for 58% (roots), 73% (stalk), and 80% (ear) (Table 3) Fecenko et al. (1997) reported a decrease in Cd contents up to 80% in barley straw and up to 50% - in barley grain after applying humic substances to soil.

Lead. There was no significant difference observed in the content of Pb in stalks of rye cultivated in soils treated with the "Rekulter" (Table 4). The Pb contents in roots and ear of the plants grown in the soil amended with the highest dose of Rekulter were 63% and 53%, respectively, lower as compared to the control plants.

Zinc. At the highest dose of the "Rekulter", Zn contents in both roots and ear of rye were about 50% lower as compared to control (Table 5). The content of Zn in the stalk dropped down to 20% of the control. Veeken (1998) concluded that heavy metals are adsorbed to the organic particles. This relationship, in our experiment, is shown by the dose of Rekulter (which is very rich in humic acids) and contents of heavy metals in plants.

The obtained yields of fresh and dry biomass are shown in Table 6. The highest yield of rye was observed with the highest dose of the "Rekulter". This indicates that application of the "Rekulter" creates beneficial conditions for the plant growth.

Table 6. Yield of rye(g per pot).

Dose of Rekulter [g per pot]	Fresh mass roots	Dry mass stalk
0	256.25	72.5
180	467.,5	158.7
360	488.75	155.0
720	521.77	193.7
LSD α=0.05	103.8	48.0

LSD α=0.05 – least significant difference, P=0.05

Table 7. Bioacumulation indexes (BI) of Cd in rye dry mass.

Dose of Rekulter [g per pot]	*Part of rye*		
	roots	stalk	ear
0	0.26	0.43	0.079
180	0.29	0.14	0.041
360	0.28	0.16	0.039
720	0.13	0.14	0.020
LSD α=0.05	0.008	0.006	0.0037

LSD α=0.05 – least significant difference, P=0.05

The calculated bioaccumulation (BI) indexes of Cd, Pb, Zn are given in Tables 7 - 9. They are indicative of mobility of Cd, Pb and Zn in soils and of their availability to plants. The BIs decreased along with an increase in the application rate of the "Rekulter" and reached the smallest values at the highest dose of the brown coal amendment (720g per pot). These trends were observed for all parts of rye and all metals studied. Although, absorption of heavy metal is very high (metal concentrations in roots), the translocation of metals to other plant parts decreased (metal concentrations in stalk and ear).

The results obtained in this study are in agreement with reported data on heavy metal hazards in abandoned mining area and metals accumulation in different parts of plants (Mocko and Waclawek, 2004). Of particular importance is that the BI for Cd were the smallest (compared to stalk and roots) for rye ear which is used as the food for animals. In general, for all heavy metals studied and for all parts of rye, the lowest BI indexes were observed at the highest application rates of the “Rekulter”.

Table 8. Bioacumulation indexes (BI) of Pb in rye dry mass.

Dose of Rekulter [g per pot]	*Part of rye*		
	roots	stalk	ear
0	0.070	0.030	0.030
180	0.050	0.030	0.028
360	0.049	0.029	0.029
720	0.020	0.029	0.027
LSD α=0.05	0.0019	0.0053	0.0055

LSD α=0.05 – least significant difference, P=0.05

Table 9. Bioacumulation indexes (BI) of Zn in rye dry mass.

Dose of Rekulter [g per pot]	Part of rye		
	roots	stalk	ear
0	0.79	2.59	1.08
180	0.79	0.,83	0.64
360	0.59	0.87	0.61
720	0.38	0.57	0.56
LSD α=0.05	0.074	0.042	0.048

LSD α=0.05 – least significant difference, P=0.05

4. CONCLUSIONS

Amendment of the soil selected for this study (Haplic Luvisols) with the "Rekulter" (a brown coal-derived preparation) significantly decreased soil acidity and increased the content of organic carbon in the soil. The "Rekulter" treatments were efficient for immoblizing cadmium, lead and zinc. The efficiency of metal immobilization was significantly higher for cadmium and zinc as compared to lead, and depended on application rate of the “Rekulter”.

The brown coal preparation treatments caused a remarkable decrease in the contents of Cd, Pb and Zn in the test-plants (rye). The positive influence was visible for all doses of the “Rekulter” applied to the soil. Accumulation of Cd, Pb and Zn in the different parts of rye plants depended on the “Rekulter” dose and decreased along with an increase in application rate of the "Rekulter". At the highest dosage used (720 g per pot, the content of heavy metals in rye was the smallest for all tested parts. The largest reduction in uptake was obtained for Cd, as compared to Pb and Zn. The BI indexes for Cd, Pb, and Zn were proportional to the contents of bioavailable (EDTA-extract) in the soil. The smallest BI indexes were observed at the highest dosage of Rekulter. This is indicative of heavy metals immobilisation by humic substances from brown coal.

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USE OF ACTIVATED CARBON FOR SOIL BIOREMEDIATION

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Abstract: The use of activated carbon may help overcome the toxicity of organic pollutants to microbes and plants during soil bioremediation. Experiments were conducted with 3,4-dichloroaniline (DCA), 2,4,6-trinitrotoluene (TNT), and polychlorinated biphenyls (PCB) to demonstrate that activated carbon (AC) can reduce the toxicity of readily available chemicals in soil by transferring them to a less toxic soil fraction. This process results in accelerated biodegradation of DCA by inoculated chloroaniline-degrading strains. In the case of TNT, the AC promotes strong binding through accelerated microbial reduction of its nitro groups and catalytic chemical oxidation of the methyl group and polymerization or binding of the products formed. Degradation of PCB in soil is rather slow. However, amendment with AC leads to a sharp reduction of extractable PCB, mostly due to strong binding to the adsorbent. The introduced AC was shown to maintain a low content of toxicants in soil solution, creating favorable conditions for plant growth, while in the unamended soils plants die or are highly inhibited. Biotests with *Daphnia magna* also demonstrated a sharp reduction of the toxicity of these contaminated soils in the presence of AC.

Key words: 3,4-dichloroaniline; 2,4,6-trinitrotoluene; polychlorinated; biphenyls; bioremediation; soil; activated carbon

1. INTRODUCTION

Various adsorbents are used for decontamination of soil and water. Among others, activated carbon (AC) is one of the best adsorbents for many

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organic chemicals because of its hydrophobicity, high specific surface (800-1200 m^2/g), and microporous structure. The average pore diameter of AC (1 or 2 nm, 0.35 nm minimum), is close to the size of monoaromatic molecules (O'Brien, 1992). This adsorbent is widely used for cleaning up drinking water and contaminated wastewater. AC has also been recommended for reducing the phytotoxicity of many herbicide residues and other chemicals in agricultural soils (Strek et al., 1981; Mukhin et al., 1995).

During soil bioremediation AC is applied for removal of volatile organics (petroleum hydrocarbons, chlorinated solvents, polychlorinated biphenyls (PCB), pentachlorophenol) and for adsorption of organic contaminants from the pumped underground waters (Khodadoust et al., 1999; Pradhan and Srivastava, 1997). One of the most interesting uses of AC is direct application to soil.

Despite the great advantages of bioremediation, this approach is now used in only about 5% of all soil treatments. One of the reasons for the low effectiveness of soil bioremediation is the high toxicity of chemical contaminants to microbes and plants. This phenomenon often restricts use of this method for highly contaminated soils. Amendment of soil with natural adsorbents can help to overcome this problem. We suggested applying AC for accelerated bioremediation of highly contaminated soil and successfully used this approach for cleaning soil after an accidental leakage of 17 tons of the herbicide propanil (3',4'-dichloropropionanilide) in the Krasnodar region of Russia (Vasilyeva et al., 1994, 1996). We also demonstrated accelerated degradation and/or detoxification of TNT (2,4,6-trinitrotoluene) (Vasilyeva et al., 2001, 2002] and PCB in soil in the presence of AC.

The objective of this paper is to compare our results with DCA, TNT, and PCB and determine mechanisms of their biodegradation and detoxification in soil in the presence of AC. Laboratory-scale experiments are described.

2. EXPERIMENTAL

Experiments with DCA were conducted with Pushchino grey forest soil (silty clay loam, C_{org} 2.1%, pH 5.5) spiked with DCA (6250 mg/kg) and historically contaminated Krasnodar meadow chernozemic soil (clay loam, C_{org} 2.8%, pH 7.1) shortly after propanil leakage (total propanil and DCA concentration was 16,000 mg/kg). The soil was amended with granulated activated carbon (GAC) "Agrosorb" (Electrostal, Russia) inoculated with *P. denitrificans* strain 3XA in an amount of 10^5 cells per g soil. The strain can grow on chloroanilines (including DCA, up to 150 mg/L) as a sole source of carbon, energy, and nitrogen. The soil samples were incubated at 28°C. The release of chloride may be used to measure DCA degradation rate because the rate-limiting process during its utilization by *P. denitrificans* is ring dihydroxylation and splitting, followed by rapid degradation of the formed dichloromuconic acid with simultaneous accumulation of chloride (Surovtseva et al., 1996). The population of chloroaniline-degrading bacteria was determined in soil through their specific activity using a kinetic method (Vasilyeva et al., 1995).

Degradation of TNT was studied in Sharpsburg (Nebraska, USA) soil (silty clay loam, C_{org} 3.4%; pH 7.5) spiked with [^{14}C]TNT (500, 1000 and 2000 mg/kg). These soils were amended with powdered activated carbon (DARCO), particle size 35 µm (0.25, 0.5 and 1% w/w). Corn (*Z. mays* L.) seedlings were planted in pots containing the soils and placed into a controlled environment chamber at 22°C. Corn was used as a quickly growing plant having great biomass.

The experiments with PCB were conducted on a sample of historically contaminated soil located near a capacitor plant in Serpukhov (Moscow region, Russia), which had been in operation for 25 years. Use of PCB until 1990 has resulted in significant contamination of the soil, and there are many contaminated areas (1 - 10,000 mg PCB/kg). To date, no effective approaches for remediation of these soils have been found. Samples of surface soil were obtained from two locations: A (histosol, sandy loam, pH 7.3, C_{org} 14.1 %) and B (alluvial, sandy loam, pH 7.3, C_{org} 2 %), initial PCB concentrations were 4024 and 1700 mg/kg, respectively. The soils were sieved through a 2 mm sieve, amended with GAC "Agrosorb" and incubated in pots under outdoor conditions beginning June, 2003. Optimal soil moisture was maintained by regular watering with distilled water as needed. The pots were seeded with white clover (*Trifolium repens* L.) after 400 d, and the clover was harvested and weighed at six months after seeding. Clover was used as a well growing in temperate climate plant having developed rootage.

A fractionated analysis of the soil was carried out to determine water extractable, solvent extractable, and unextractable fractions of the chemicals. These fractions of organic chemicals are considered as readily available (RA), potentially available (PA), and practically unavailable to microbial degradation or bound (BD), respectively.

The DCA-contaminated samples were extracted with distilled water (1:200) (RA fraction), then three times with acetone (1:100) (PA fraction), and finally the hydrolysable bound DCA was determined through hydrolysis in boiling 5 mM NaOH. Total degradation of DCA was determined from the accumulation of chloride in the incubated soil.

The [^{14}C]TNT contaminated soil samples were extracted with 3 mM $CaCl_2$ (1:5) (RA fraction), then the PA fraction was extracted with acetonitrile (1:10) by sonicating, and finally the BD fraction of TNT was measured as bound ^{14}C by combusting the sample in a sample oxidizer and measuring $^{14}CO_2$. The rate of [^{14}C]TNT mineralization was determined from the amount of $^{14}CO_2$ released.

The soil samples contaminated with PCB were analyzed by the method officially accepted in Russia and described in Bobovnikova et al. (2000). This fraction was considered as extractable PCB, which contains the first two fractions (RA and PA). For this purpose the soil was extracted three times with hexane:acetone (4:1). In addition, the bound PCB was determined by extraction with boiling toluene.

Concentrations of the chemicals and their products in the extracts were determined by HPLC, GC, or GC-MS. The DCA concentration in extracts was determined with HPLC Liquohrome "Radelkis" with UV-VIS and a Nucleosil column (Keystone Scientific, Bellefonte, PA, USA). The mobile phase was an isocratic mixture of 1.38 g CH_3COONa and 20 ml ice CH_3COOH in 1l of water and a methanol mixture (30:70, v/v), the pH of the mixture was 3.3. The TNT and products were determined with HPLC (Shimadzu, Kyoto, Japan) equipped with the Betacil NU column (Keystone Scientific, Bellefonte, PA, USA). The mobile phase was an isocratic mixture (50:50, 40:60 or 35:65 v/v) of acetonitril and deionized water acidified with H_3PO_4 to pH 3.3. The accumulation of DCA and TNT products was confirmed with GC-MS-analysis (HP 6890 gas chromatorgaph with an HP 5972A mass-selective detector, Hewlett-Packard, San Fernando, CA, USA). Separations were obtained using a 5% cross-linked phenyl methyl siloxane capillary column HP-5 with a 0.25 mm inner diameter 30 m length, 0.25-mm film thickness. The carrier gas was helium at 1 ml/min. Analysis of extracts of PCB was carried out with GC-MS Finnigan MAT SSQ 7000. The column and carrier gas conditions were the same as previously described. During PCB analyses deuterated naphthaline and phenanthrene were used as the internal standards. All the details are described in (Bakhaeva et al., 2001; Vasilyeva et al., 2001).

The toxicity of the soil samples was determined through biotests with *D. magna* using the approach described in van Gestel et al. (2001). In addition, the phytotoxicity of these soils was determined on germination and growth of cress (*Barbarea* spp.) seedlings, as well as on the biomass of corn (*Z. mays* L.) or clover plants grown in the soil after harvesting. Detailed descriptions of the experiments and analyses appears in Vasilyeva et al. (1996, 2001, 2002) and Bakhaeva et al. (2001).

All the experiments were taken in three replicates, and statistical analyses of the results was carried out with the help of the computer programs Excel and SigmaPlot 2001.

3. RESULTS AND DISCUSSION

Degradation of DCA. DCA is a persistent and toxic metabolite of arylamide herbicides, including propanil, which is used on rice and cotton (*Gossypium* spp.). In contrast to readily degradable propanil, DCA can be degraded by chloroaniline (CA)-degrading microorganisms which are only prevalent in soils chronically contaminated with these contaminants. We isolated several bacterial strains growing on CA (including DCA) as a sole source of nitrogen, carbon, and energy from rice (*Oryza* spp.) paddy soils (Surovtseva et al., 1984, 1996) and two pathways (Figures 1, 1a and 1b) of DCA degradation have been established. However, maximal DCA concentrations in the media which maintain growth of the bacteria do not exceed 150 mg/L, which is substantially below the DCA solubility (300-400 mg/L). Thus these bacteria will be strongly inhibited or die in highly contaminated soils.

The next pathway of DCA transformation (2) is oxidation by microbial or plant peroxidases, resulting in accumulation of a persistent metabolite, tetrachloroazobenzene (TCAB), having carcinogenic and mutagenic properties [Bordeleau and Bartha, 1972].

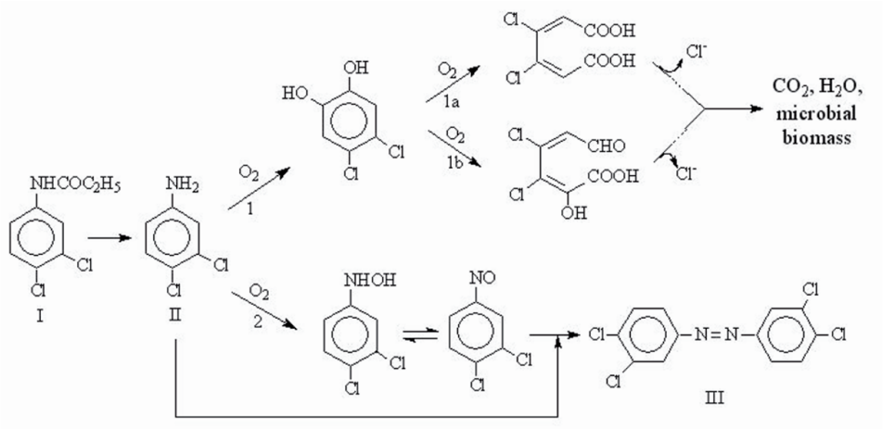

Figure 1. Scheme of microbial degradation of propanil (I) followed by DCA (II) degradation by CA-degrading bacteria (1) and DCA transformation to tetrachloroazobenzene (III) by plant and microbial peroxidizes (2). (Bordeleau and Bartha, 1972; Surovtseva et al., 1984, 1996).

The experiments indicated that total extractable DCA sharply decreased in soil inoculated with the *P. denitrificans* strain and amended with 1% AC in comparison to the unamended control (Figure 2A). Almost all of the degraded DCA was mineralized by the introduced bacteria, confirmed by the liberation of chloride ions (72% of total). The water-extractable fraction of

DCA was readily available (RA) to degrading microorganisms and its degradation was accompanied by slower degradation of the solvent-extractable fraction, which can be considered as the potentially available fraction (PA). After a 140-d incubation, only 3% of the total extractable DCA remained in the amended samples compared to 60% in the unamended control in which no chloride ion accumulated. The final accumulation of bound hydrolysable DCA (BD) in the experimental soils did not exceed 4%, while the disappearance of DCA in the control samples was mostly due to formation of BD and probably a nonhydrolysable bound fraction as well as some metabolites. The concentration of TCAB in the unamended soil reached 150 mg/kg, while its concentration in the amended soil did not exceed 0.02 mg/kg. Inoculation of *P. denitrificans* into the soil at the lower AC dose (0.1%) as well as a separate introduction of the strain or AC did not result in DCA degradation. The introduced bacteria died in the these samples while their number in the optimal samples was maintained at 10^5-10^7 cells/g for several months, then decreased to about 1 cell/g.

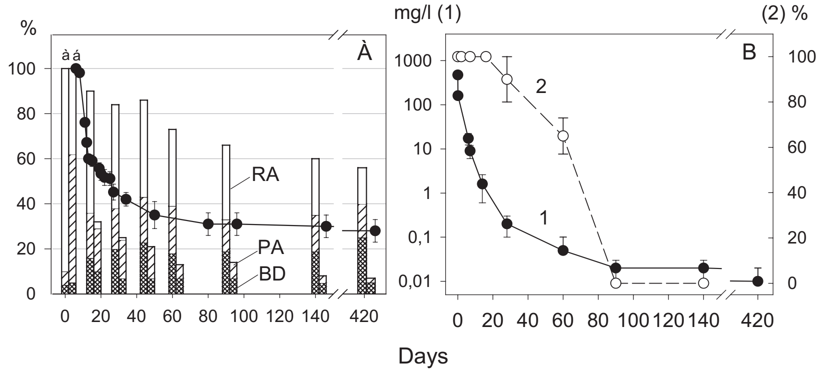

Figure 2. A – Content of DCA fractions in control unamended (a) and in the experimental (b) soils amended with 1% G AC and inoculated with *P. denitrificans* and estimated remaining DCA based on its dechlorination in the experimental soil (curve). B – Content of DCA in soil suspension (1:1) – (1) and mortality of *D. magna* in biotests – (2) for experimental samples. Additional details appear in the text.

The DCA concentration in soil solution (Figure 2B) was reduced to 50 mg/L on the second day after introducing 1% AC and to 0.01 mg/L at the end of the experiment, while the concentration in other samples was 300 to 350 mg/L. Biotests with *D. magna* indicated that biotoxicity of the experimental soil was sharply reduced after three months (Figure 2B) and no biotoxicity was detected in the decontaminated soil at the end of the experiment.

Similar results were obtained with Krasnodar soil historically contaminated with propanil. After two or three months this soil contained mostly DCA, and its fractional distribution-was similar to the spiked soil.

Degradation of TNT was also accelerated after mixing soil with AC, and the largest effect was at the highest TNT concentration (2000 mg/kg). The readily available TNT almost disappeared within 6 h after AC amendment, and the concentration of solvent-extractable TNT reached 134 mg/kg after four months of incubation. This value in unamended soil was 793 mg/kg, and about a half of the extractable TNT was readily available (Figure 3A).

Only 1 or 3% of the initial TNT was transformed to reduction products, mostly mono-amino and mono-hydroxylamino-derivatives and small amounts of tetranitroazoxybenzene (Figure 4, III) in amended and unamended soils, respectively. These products accumulated substantially faster and then diminished in the amended soil. In addition, small amounts of oxidation products (2,4,6-trinitrobenzyl alcohol, 2,4,6-trinitro-benzaldehyde and 1,3,5-trinitrobenzene) temporarily accumulated in the AC-amended soil samples.

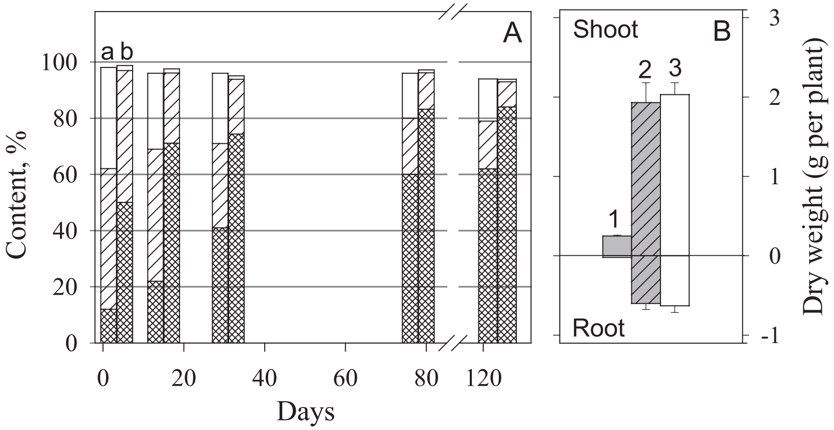

Figure 3. A – Content of TNT fractions in unamended (a) and AC-amended (b) soils with initial TNT concentration 2000 mg/kg. B – Biomass of corn plants grown in this TNT-contaminated soil, unamended (1) and AC-amended (2) in comparison to uncontaminated-soil (3). The legend is similar to Figure 2.

Only minor amounts of ^{14}C-TNT (1-2%) were mineralized to $^{14}CO_2$ in both treatments. Almost all of the extractable ^{14}C-TNT that was lost was detected as bound ^{14}C. However, the process of TNT binding occurred much faster and was more extensive in the AC-amended soil compared to the control (final content was 85 and 62%, respectively). The bound ^{14}C-products were very resistant to solvent extraction. Practically no ^{14}C-products were released from the amended soil after three or four sequential extractions with acetonitrile.

The AC reduced the phytotoxicity of TNT in the soil. While corn plant growth was severely inhibited in TNT-contaminated soil, growth characteristics in AC-amended contaminated soil were similar to the AC-amended, uncontaminated control (Figure 3B). Initial TNT concentrations in the solutions of amended and unamended soils were 5 and 80 mg/L, respectively. The results corresponded to the minimal phytotoxic concentration of TNT in solution (5 mg/L) determined in experiments with corn and tall fescue (*Festuca arundinacea* Shreb.) seedlings grown in hydroponic culture in the presence of TNT. Tissues of harvested corn plants contained no more than 0.5% of the initially added ^{14}C, demonstrating low accumulation of TNT and its products in corn plants growing in contaminated soil amended with AC. Microbial toxicity of TNT was indicated by a greater than 50% decrease in platable heterotrophic microorganisms in soils containing 1000 or 2000 mg TNT/kg. This contrasted with the greater number of microorganisms in all TNT-contaminated soils amended with AC, which was not influenced by the initial TNT concentration in soil.

The results suggest the next scheme of TNT detoxification in soil in the presence of AC (Figure 4). The AC promoted strong binding of TNT to the soil matrix. TNT binding occurred in two steps. First, some of the adsorbed TNT was rapidly bound to AC through catalytic oxidation and polymerization of the oxidized products.

Then the remaining TNT that was reversibly adsorbed to AC was microbially (and chemically) transformed to reduction products, which were bound to soil humus and likely polymerized with the TNT oxidation products. TNT reduction occurs via a common metabolic pathway, which can be mediated by different soil microorganisms in the presence of other substrates under anaerobic or aerobic conditions (Rieger and Knackmuss, 1995). TNT oxidation in the amended soil provides evidence of the strong oxidative properties of AC. It likely catalyzes formation of active oxygen-containing species (OH-radical and others) on the AC surface, including its intraporous spaces (Bansal et al., 1988). These radicals can promote formation of 2,4,6-trinitrobenzene radicals (II). Phenols were shown to bind to AC through a similar oxidation mechanism (Tessmer et al., 1997).

Another possible mechanism of TNT binding to the AC is π–π bonds between TNT molecule and the graphene (polycyclic aromatic) surface of AC as demonstrated by Zhu and Pignatello (2005). The bound TNT products were shown to be nontoxic to heterotrophic soil microorganisms. Additional details can be found in Vasilyeva et al. (2001, 2002, 2003).

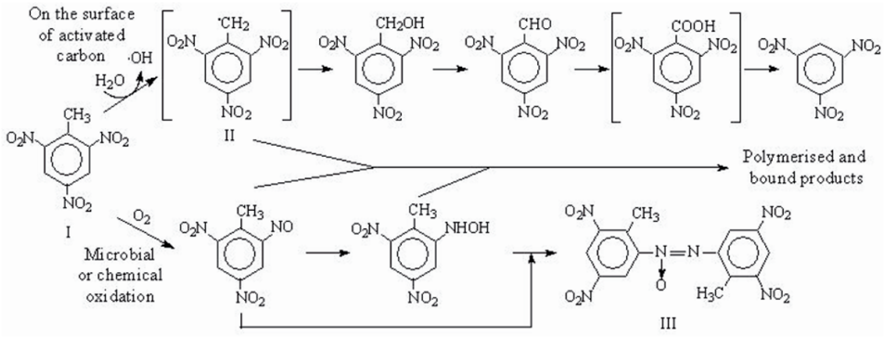

Figure 4. Suggested scheme of microbial and chemical oxidation and binding of TNT (I) in soil amended with activated carbon (see text).

Degradation of PCB. Microorganisms can degrade PCB in soil and sediments, however that process is rather slow. It is now commonly accepted that highly chlorinated PCB first should be transformed to less chlorinated homologues (mainly tri- or tetrachlorinated) through reductive dechlorination (Bedard and Quensen, 1995). Then the less chlorinated PCB congeners can be slowly degraded by aerobic soil microorganisms via pathways similar to those outlined in Figure 5 (1 and 2, respectively).

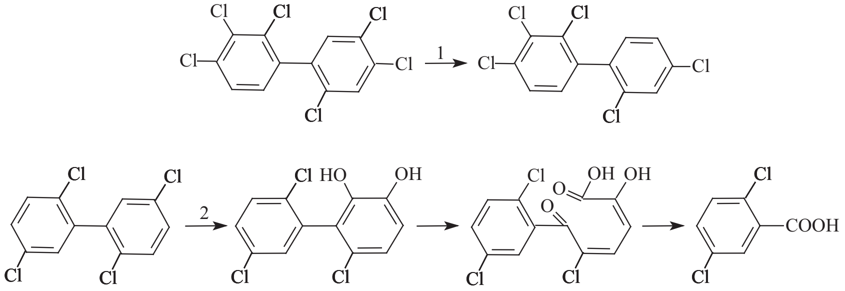

Figure 5. Reductive dechlorination of 2,2',3,4,4',5-hexachlorobyphenyl (1) and oxidative degradation of 2, 2', 5, 5'-tetrachlorobyphenyl by Alcaligenes sp., JB1 (2). (Bedard and Quensen, 1995; Komancova et al., 2003)

Until recently no strains have been isolated that can use any polychlorinated biphenyl (with more than one chlorine substitute) as a sole carbon and energy substrate. PCB can be degraded by microorganisms under co-oxidation conditions only, and degradation rate of highly chlorinated PCB congeners is very low (Boria et al., 2005).

Our experiments confirmed extremely high persistence of PCB in both soil samples (Figure 6A and 6B). At the end of the experiment (640 d), total

PCB was reduced by 20 and 62% in soils A and B, respectively. Mixing soil B with of 0.5% AC had little effect on the dynamics of PCB concentration in the soil. Amending soil A with 7% AC substantially reduced the concentration of extractable PCB, but its subsequent disappearance was slower.

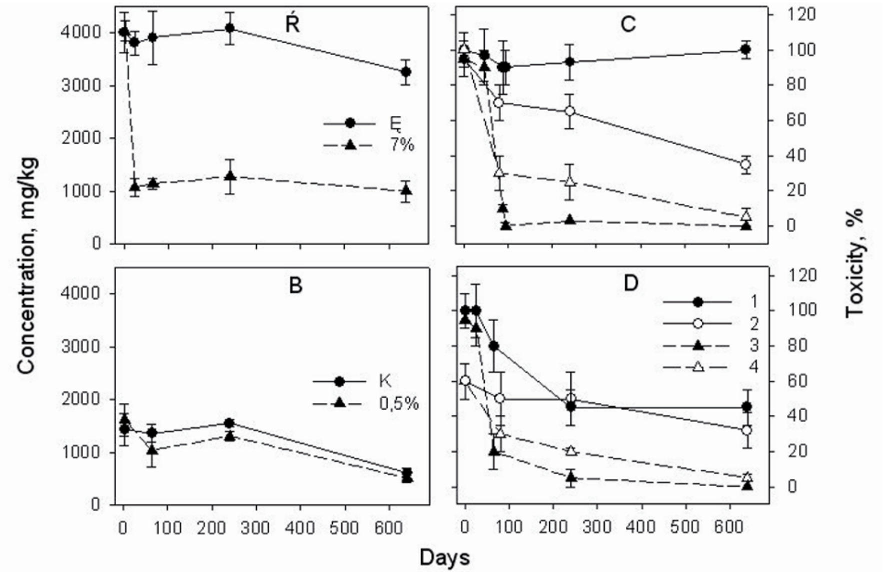

Figure 6. Dynamics of PCB in soil A - (A) and B – (B) amended with AC (7 and 0.5%) in comparison to unamended controls (K) as well as biotoxicity (1, 3) and phytotoxicity (2, 4) in control (1, 2) and AC-amended (3, 4) soils A – (C) and B – (D).

The AC amendment reduced the toxicity of these highly contaminated soils (Figure 6C and 6D). While unamended soil samples (both A and B) remained highly toxic to daphnia and plants, the toxicity of the amended soils was significantly reduced shortly after mixing with AC, and almost no toxic effects were found by phytotests and biotests with *D. magna* in amended soils A and B after 2 or 8 months. Only a small toxic effect was detected in chronic tests with *D. magna* in soil A amended with 7% AC. In addition, the biomass of white clover grown in the AC-amended, PCB-contaminated soils (both A and B) was similar to those grown in pure control soil, while the plants did not grow or were greatly inhibited in unamended soils A and B (similar to experiments with TNT).

A study of PCB congeners indicated that mostly tri-, tetra- and pentachlorinated and small amounts of di-, hexa- and heptachlorinated homologues were initially present in the soils (Figure 7). This is related to the fact that less chlorinated biphenyls (of the Aroclor 1242 type) were used at the capacitor plant. During the 21-month experiment, only the content of homologues with three and four chlorines was reduced in soil A: in the unamended control by 76 and 15%, and in amended samples by 92 and 72%, respectively (Figure 7A). In soil B these values for the 3-, 4-, and 5-chlorinated homologues in unamended soils were 80, 54, and 55%, respectively, and 82, 69 and 66% in amended soils, respectively (Figure7B).

The disappearance of the main PCB homologues extracted from soil by a conventional extractant mixture (hexane-acetone) was faster in the presence of AC, especially at its higher dose in soil A. However, detailed analyses indicated that a substantial amount of PCB was bound to AC, and actual degradation rates were comparable in AC-amended soil and in the unamended control.

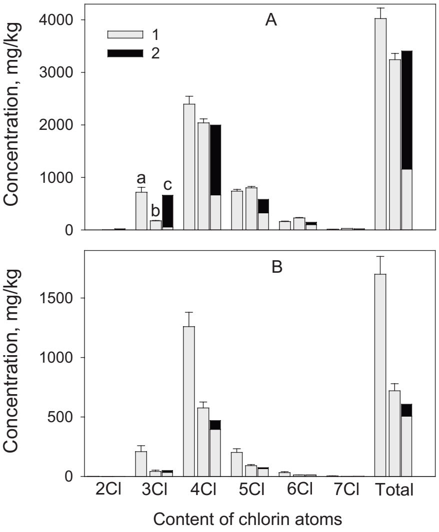

Figure 7. Content of PCB homologues at the start (a) and end of incubation (b, c) in unamended (b) and AC-amended (c) soil A (A) and B (B).

Interestingly, the relative amounts of bound PCB homologues decreased proportionally with an increasing number of chlorines in the molecules. For AC-amended soil A, the rate of bound di-, tri-, tetra-, penta-, and heptachlorinated biphenyls reached 93, 86, 54, 29, 21 and 17% of the total amount (extractable and bound) of each homologue group in the sample, respectively. For soil B these values were 50, 35, 15, 12 and 11%, respectively (there were no PCB congeners with seven chlorines). This corresponds to the proportion of non-ortho-chlorinated and mono-ortho-chlorinated congeners having planar structure among all possible congeners of the same homologue group. These values are 80, 50, 35, 20, 11 and 6% for di-, tri-, tetra-, penta-, and heptachlorinated biphenyls, respectively. Results corresponded to the increased sorption of planar PCB congeners by soot (Bucheli and Gustafsson, 2003).

The most probable mechanism of PCB binding to AC is formation of π–π-bonds with the graphene surface of the adsorbent. These bonds can be especially strong when the planar PCB molecules penetrate the nearest nanopores where their molecules may form π–π-bonds with both walls of the slit-like pores of the carbon. Nonplanar molecules of PCB having two and more ortho-chlorines should form weaker π–π -bonds with the AC surface, and these bulkier molecules may have less potential to penetrate into the narrow pores.

4. CONCLUSIONS

Soil amended with AC accelerated detoxification and transformation of all three chemicals studied; however the mechanism differed depending on the chemical. An adequate dose of AC reduced the content of readily available DCA and TNT and their concentration in soil solution, thus creating favourable conditions for degrading microorganisms and plant growth. Most of the DCA was physically adsorbed to AC and remained largely available for utilization by CA-degrading bacteria. This resulted in degradation of most of the DCA and a tremendous reduction in biotoxicity of the soil.

In the case of TNT, activated carbon (AC) promotes strong binding through accelerated microbial reduction of its nitro groups and catalytic chemical oxidation of the methyl-group and polymerization or binding of the products formed. Degradation of PCB in soil is rather slow. However amendment with AC leads to a sharp reduction of extractable PCB, mostly due to strong binding to the adsorbent. The introduced AC was shown to maintain a low content of toxicants in soil solution, creating favourable conditions for plant growth, while plants died or were greatly inhibited in unamended soils. Biotests with *D. magna* also demonstrated a sharp reduction of the toxicity of these contaminated soils in the presence of AC. Our research results demonstrate the potential of AC to decrease toxicity and promote biodegradation processes in highly contaminated soils.

ACKNOWLEDGEMENTS

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ADSORPTION OF ANIONS ONTO SOL-GEL GENERATED DOUBLE HYDROUS OXIDES

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Abstract: The original sol-gel method developed at the Institute, which allows the sorbents to be obtained from non-traditional, easily available, and cheap raw materials, was used to synthesize the novel ion exchangers based on double hydrous oxides ($Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$ and $2Al_2O_3 \cdot ZnO \cdot xH_2O$), which were tested for their adsorption characteristics towards the anions ($H_2AsO_4^-$, $H_2PO_4^-$, F^- and humic acids) from simultaneous solutions. Adsorbents were characterized by potentiometric titration and poremetrical characteristics. The technologically attractive effect of pH on the anions sorption on the developed double hydrous oxides, which are able to work in the pH range from 3 to 10, was found. Isotherms for these anions were obtained and kinetics was investigated. Mechanism of fluoride adsorption to the surface of the model cluster of the synthesized sorbent and geometry of the cluster itself were modeled with quantum-chemistry HyperChem7 program using the PM3 method.

Key words: inorganic ion exchangers; adsorption; anions; isotherms; quantum chemistry modeling

1. INTRODUCTION

The increased environmental pollution in the European countries and in the whole world caused by various anthropogenic activities and natural events led to a deterioration of the quality of drinking water and requires the removal of a variety of pollutants from natural waters and waste water streams, food stuff purification, and the development of pollution prevention

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technologies. In order to be able to meet the new standards more effective and inexpensive purification methods are needed.

The majority of investigations in the field of elimination of inorganic contaminants in water treatment are devoted to cationic contaminants. However, many toxic elements occur in aqueous solutions in anionic forms including those of As, P, F, Sb, Se and Cr that need to be removed.

Drinking water pollution by arsenic is a serious problem for many parts of the world described in numerous publications (e.g. WHO, 1993; FRAEA, 2002). To protect the population most countries have introduced a standard of 10 µg/L for drinking water. Different methods have been applied to the removal of arsenic from natural and waste waters, among them (1) ion exchange using inorganic sorbents with or without regeneration turned out to be one of the most efficient, cost effective and promising processes for industrial application along with (2) coagulation by salts of iron followed by micro-filtration and (3) oxidative removal by iron and/or manganese in the raw water at sufficient concentrations (Leist et al., 2000; EPA, 2000). The concentration of the anions of other elements (P, F, Sb) in drinking water is strictly controlled as well (WHO, 1993).

The presence of trace amounts of phosphate (even less than 1 mg/L) in treated wastewater is often responsible for eutrophication; particularly in lakes and slow moving rivers. As a result legislative requirements and recommendations were issued (e.g. the EU Wastewater Directive, 1991 http://europa.eu.int/comm/environment/water/water-rbanwaste/directiv.html) to reduce phosphate concentrations in domestic and industrial waste waters. There is a wide range of technologies to remove phosphorus (P) from wastewater, including chemical precipitation (CP), biological phosphorus removal (BPR), crystallization, novel chemical precipitation approaches as well as adsorption or ion exchange processes (Ruixia et al., 2002).

Fluoride is an essential micro-nutrient for living beings, however, with a narrow range of tolerance. Greater concentrations may lead to different forms of fluorosis (Zheng, 1994). Wastewaters from phosphate fertilizer plants may contain up to 2% of fluoride (Ryżycka and Grobelny, 1998). Increased levels of fluoride can also be found in effluents from the fluorine industry, glass etching (Sahu et al., 1998), microelectronics and in ground water around aluminum smelters (Arnesen, 1998). High concentrations of fluoride and boron occur in mineral waters of Ukrainian Carpathian mountains, Czech Republic mineral water (Karlovy Vary), etc. Major methods for removal of fluoride and bromide ions in large scale are precipitation and coagulation, as well as sorption and ion exchange.

Selective ion exchange without or with regeneration of the materials was found as one of the most promising methods to solve the problem of removal of arsenic, fluoride and phosphorus species. This method is especially useful if the element has to be removed from a multicomponent system, from which only the harmful components should be eliminated. Usual treatment processes like chemical precipitation are often not effective enough, membrane processes are by no means selective enough and for conventional sorbents the competitive uptake of species from the background composition of the waters is often too great to allow a sufficiently selective sorption of the target species (WHO, 1993; Leist et al., 2000; EPA, 2000). An effective elimination of trace components without substantial change of the background composition of water needs specific sorbents of sufficient selectivity for the target species. Many sorbents have been tested, (activated alumina, gibbsite, aluminium-loaded materials, lanthanum compounds, fly ash). Fe(III)-containing materials (goethite, hematite, iron-coated sand, iron(III)-loaded resins) turned out to be successful for arsenic species removal, whereas polymeric exchangers revealed a much worse performance than inorganic sorbents (Zhuravlev et al., 1999).

Traditionally, inorganic adsorbents have been obtained by hydrolysis (precipitation) of salts. Recently, a sol-gel method based on controlled hydrolysis of expensive alkoxy compounds of the corresponding metals has been used more extensively. At the Institute for Sorption and Problems of Endoecology, National Academy of Sciences of Ukraine an original sol-gel method of synthesis has been developed (Zhuravlev et al., 1999; Chubar et al., 2005) that allows production of spherically granulated sorbents from inexpensive raw materials (simple inorganic salts of the corresponding metals, mineral acids and alkalis).

The main objectives of this work were using the original sol-gel method to synthesize the novel inorganic ion exchangers based on mixed hydrous oxide of iron, aluminium and zinc, to investigate adsorption of arsenic, phosphorus, fluoride and humic acids in batch conditions (pH effect, isotherms and kinetics) and to use the quantum-chemistry HyperChem7 program (PM3 method) to optimize the model cluster of the obtained sorbent and propose the mechanism of fluoride adsorption.

2. MATERIALS AND METHODS

2.1 Synthesis of the Sorbents and Their Textural Characteristics

The chloride salts of iron and aluminium ($FeCl_3 \cdot 6H_2O$, $AlCl_3 \cdot 6H_2O$ and $ZnCl_2$) and an aqueous solution of ammonia (25%) and some other soft neutralizers have been used for the synthesis of the novel ion exchangers. All reagents used were of analytical grade. The raw materials (mixture of the initial salts) were subjected first to preliminary neutralization. The streams of these precursors (sols like Al-Fe-$(OH)_5Cl$) were dispersed dropwise in undecane and the small drops formed in undecane fell down into the ammonia solution for $Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$ and to a solution of $NaHCO_3$ for $2Al_2O_3 \cdot ZnO \cdot xH_2O$, where sols were transferred into gels (hardening) forming spherical granules with little deformation.

X-ray analysis confirmed that the materials were amorphous. Their textural characteristics are shown in Table 1.

Table 1. Some textural characteristics of $Fe_2O_3 \bullet Al_2O_3 \bullet xH_2O$ and $Al_2O_3 \bullet ZnO \bullet xH_2O$.

Oxide, the elements relative M content	Specific surface area, (S_s), m^2/g	Pore volume (V_s) in water, cm^3/g	Pore volume (V_s) in benzene, cm^3/g	Moisture, mass %
$Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$	396	0.15	0.28	20.4
$Al_2O_3 \cdot ZnO \cdot xH_2O$	33	0.14	0.54	19.4

2.2 Potentiometric Titration

Cation and anion exchange capacities of the investigated sorbents (regarding H^+ and OH^- ions correspondingly) were studied by potentiometric titration as described by Grissbach (1963) and Hiemstra and van Riemsdijk (2000). Typically, one gram of ion exchanger was introduced into the flasks containing 50 cm^3 of 0.1 M NaCl, then a definite volume of 0.1 M NaOH or HCl was added. The flasks were placed in the temperature controlled orbital shaker for 48 hours, then the equilibrium pH was measured. The calculation of ion exchange capacity ***q***, mM_c/g) was carried out using equation 1:

$$q = \frac{C(V_1 - V_2)}{1000 \times m} \tag{1}$$

where *C* (M/mL) is the concentration of the acid/alkali, V_1 (mL) is the volume of added NaOH/HCl in the experiment at a defined value of pH, V_2 (mL) is the volume of added NaOH/HCl in the blank experiment at the same pH and *m* (mg) is the mass of the sorbent.

2.3 Batch Sorption Experiment

Experiments were carried out in Erlenmeyer flasks placed in a temperature controlled orbital shaker (stirring speed of 150 L/min) for 48 hours as it was established previously that equilibrium was reached within 48 hours. A 200 mg portion of the sorbent was equilibrated with 100 mL of the anion solutions at 22 ± 2 ºC using a background electrolyte of 0.01 M $NaNO_3$. It was investigated separately that NO_3^- is not adsorbed by the surface of this ion exchanger and can not compete with halides for sorption sites. A suitable volume of acid (HNO_3) or base (NaOH) solution was added to adjust pH that was measured with a digital pH meter (TOA Electronics Ltd., HM-35 V). The adsorbent was removed by centrifugation, the concentration of the corresponding anion remaining in the supernatant was determined by certified methods (spectroscopy, AAS, ion chromatography).

3. RESULTS AND DISCUSSION

3.1 Ion Exchange Capacities of the Sorbents

The potentiometric titration of the two sorbents calculated according to equation {1} are shown in Figure 1. The anion exchange capacity of $Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$ (Fe-Al) and $2Al_2O_3 \cdot ZnO \cdot xH_2O$ (Al-Zn) was around 1.7 and 3.4 mM_c/g (or meq/g) respectively while their cation exchange capacities were around 1.5 and 2.7 mM_c/g (or meq/g).

The isoelectric point of Fe-Al sorbents was at neutral pH, but Al-Zn was at pH 8.8. In neutral solutions cation exchange and anion exchange functions of such ion exchangers compensate each other. It is noted that the mixed iron-aluminum oxides have similar anion and cation exchange capacity while Al-Zn oxide has a much larger anion than cation exchange capacity. These above presented data are in good agreement with the assumption that the ion exchange groups Me–OH of the hydrated oxides are capable as for anion exchange so for cation exchange according to the following scheme 1:

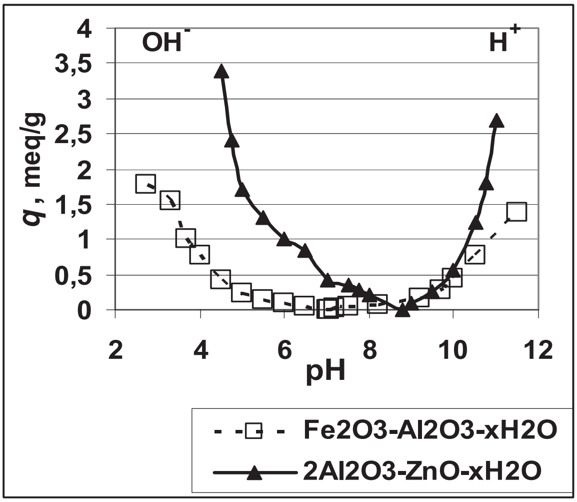


Figure 1. Potentiometric titration curves.

$$\mathrm{Me-OH + K^{+} \rightarrow Me-O^{-}K^{+} + H^{+}}$$

$$\mathrm{Me-OH + A^{-} \rightarrow Me^{+}A^{-} + OH^{-}}$$

Figure 2 (a) shows the pH effect of F^- sorption and humic acid on Fe-Al and 2Al-Zn, the initial concentration of all ions was 3 mM/L. Higher affinity of the sorbent toward fluoride is due to possible formation of insoluble iron fluoride and lower soluble aluminum fluorides. Technologically important (for drinking water treatment conditions) pH

3.2 Effect of pH

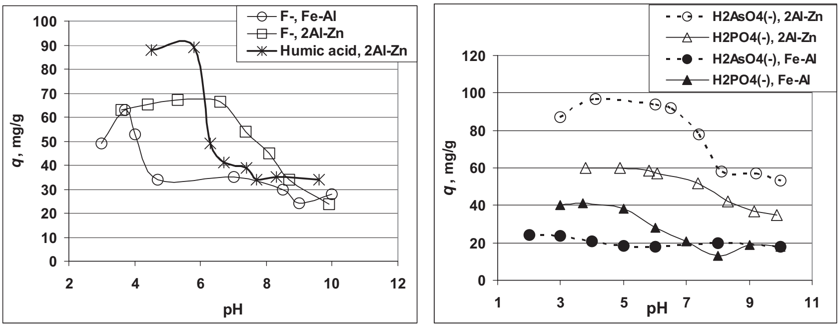


Figure 2. a) – pH effect of F^- and humic acid sorption onto Fe-Al and 2Al-Zn at the initial anion concentration of 120 mg/L; b) – pH effect of $H_2AsO_4^-$ and $H_2PO_4^-$ sorption onto Fe-Al and 2Al-Zn at the initial anion concentration of 3 mM/L.

effect on fluoride sorption on the investigated adsorbent was established. Typically, sorption of anions on individual hydrous oxides is dependent very much on pH values. For example, F^- sorption on the natural mineral Goethite (Hiemstra and van Riemsdijk, 2000) and acid treated spent bleaching earth (Mahramanlioglu et al., 2002) could take place in the acidic solutions only. Sorption of F^- decreased slowly with increasing pH and the anions were not adsorbed at all at ≥ pH 7. Fe-A and, especially 2Al-Zn, could uptake F^- (Figure 2) in the whole interval of pH from 3 to 8.5. The sorption capacity of the investigated adsorbent toward fluoride was twice as high in acidic (pH 3-4) than in the neutral and alkaline solutions. At pH 4-5 a sharp decrease of sorption from 70 to 37 mg/g has taken place on Fe-Al. At pH 7.5-8 some decrease of sorption capacity of the sorbent (near 20 mg/g) has occurred. pH < 5 is the most favorable condition for fluoride uptake due lower concentration of OH^- ions, which compete with fluoride for sorption sites. Increasing concentrations of OH^- in the solution reduce the sorption capacity toward F^-. Speciation of the investigated anion is the second reason of higher fluoride sorption at pH < 5 when it exists as [HF]. Maximal sorption capacity is at pH 4 when this is the predominant form of the anion. The adsorption of F^- can be described as an exchange reaction against OH groups of the surface.

Speciation of arsenate and phosphate, affinity of the sorbents toward the anions and surface chemistry are the main factors influencing the pH effect of these anions' adsorption. Thermodynamic calculations with the computer code MINEQL+ showed that phosphate exists in solution as H_3PO_4, $H_2PO_4^-$, HPO_4^{-2} and PO_4^{-3} at different ratios according to pH. For a solution containing only phosphate at 200 mg/L the pH of transition from the uncharged to the most charged species are 2.2, 7.2 and 12.2 respectively. It is observed that the maximum phosphate sorption occurs at pH values 3 to 5, when the singly-charged species of phosphate ($H_2PO_4^-$) dominates, then decreases with the increase of pH. Similar pH dependence was found for arsenate.

We are just beginning to study adsorption of humic acid onto new sorptive materials and removal by ion exchange, but these data are considered to be promising and worthy of further development.

Isotherms

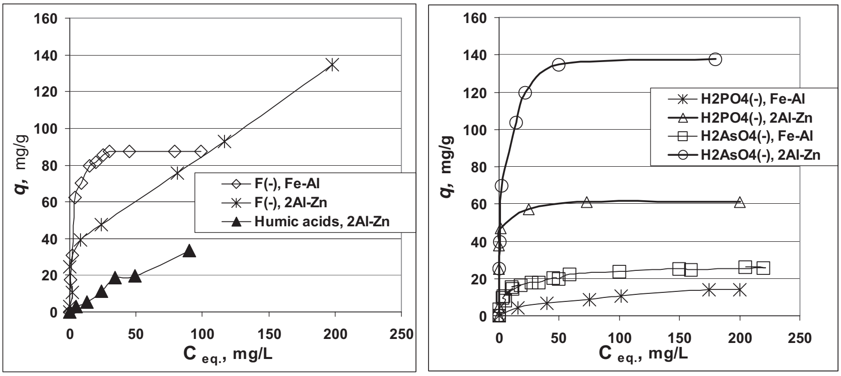


Figure 3. Isotherms of F^-, humic acid, $H_2AsO_4^-$ and $H_2PO_4^-$ sorption onto Fe-Al and 2Al-Zn at pH 7, equilibration time of 48 hours, solid concentration of 2g/L, temperature of 22 ± 2 ºC.

The isotherm of fluoride sorption (Figure 3, a) onto Fe-Al, is very close to the y axis as compared to the curve obtained for its sorption onto 2Al-Zn. This is evidence of the high affinity of the investigated adsorbent toward F^-. Sorption capacity of ion exchanger toward F^- (4.2 mM/g) was 4 times higher as compared with the sorption capacity of the new type of ion exchange fiber (Ruixia et al., 2002) and 10 times higher than of goethite (Hiemstra and van Riemsdijk, 2000). We suppose that mechanism of F^- sorption on the investigated Fe-Al is similar to that described by Hiemstra and van Riemsdijk (2000). At the lower concentration of F^- in the solution, these ions were exchanged with OH^-, coordinated on the surface, on one-charge scheme. At a higher concentration of fluoride ions, OH^- ions, coordinated on the two-point scheme, take place in the ion exchange with F^-. Re-distribution of the surface charge takes place at the formation of the surface complexes of fluoride. This is explained by change of donor-type complexes (FeOH, $Fe(OH)_2$) to proton-acceptor complexes (FeF_3). Distribution of the charge in this model is approximately equivalent to change of donor H-bond into acceptor H-bond. At very high concentrations of fluoride ions precipitation of F^- into FeF_3 occurred. Presence of high concentration of background electrolytes catalyzes this process. Isotherms of F^- sorption onto Fe-Al fit well to the Langmuir model (correlation coefficient ($r^2 = 0.9987$). Maximum sorption capacity calculated from the model was close to the experimental data (90 mg/g) and the affinity constant, which was 0.52 L/mg, is 10 times higher than analogous parameters found for acid treated spent bleaching earth (Mahramanlioglu et al., 2002).

Figure 3b shows that the sorption capacity and affinity (isotherms close to y axis) of 2Zn-Al is higher for both arsenate and phosphate as compared to the sorption characteristics of Fe-Al. Sorption capacity of phosphate and arsenate were, respectively, 18 and 23 mg/g onto Fe-Al, but 60 and 139 mg/g onto 2Zn-Al. At that, isotherms obtained on 2Zn-Al were much closer to y axis which is evidence of much higher sorption affinity of these tetrahedral anions to the surface of the ion exchanger. Such sorption capacity is very comparable to those of most recent investigations (Ruixia et al., 2002; Gao and Mucci, 2003; Patannyak et al., 2000). And, what is very valuable is that these data were obtained at the pH 7, typical for purifying waters. Most conventional adsorbents show few times lower sorption capacity toward arsenic than the investigated 2Zn-Al. Comparative sorption capacity was found for chitosan derivative (Dambies et al., 2002), but kinetics of sorption was several times slower, which makes difficult, if not impossible, to use it in industrial treatment plant conditions.

The data on the kinetics are in very good agreement with equilibrium isotherm curves. Adsorption of F^- onto Fe-Al is faster than onto 2Al-Zn, while adsorption of tetrahedral anions ($H_2PO_4^-$ and $H_2AsO_4^-$) is faster onto 2Al-Zn than onto Fe-Al. The initial concentration of all anions was 0.3 mM/L. It was found that fluoride is fully adsorbed by the surface of Fe-Al within 30 minutes and reaches 50% adsorption in a few minutes. The rate of As(V) adsorption is also very rapid onto both Fe-Al and especially onto 2Al-Zn hydrous oxides. Fifty percent of arsenate was adsorbed by the surface of 2Al-Zn within a few minutes.

3.3 Kinetics

3.3.1 Investigation of the Mechanism

It is suggested that sorption of tetrahedral anions (phosphate and arsenate) onto hydrated oxides is not only the result of ion exchange according to the following scheme 2:

$$\mathbf{{=}Fe(Al){-}OH + H_2PO_4^- \leftrightarrow {=}\ Fe(Al) - (H_2PO_4^-) + OH^-}$$

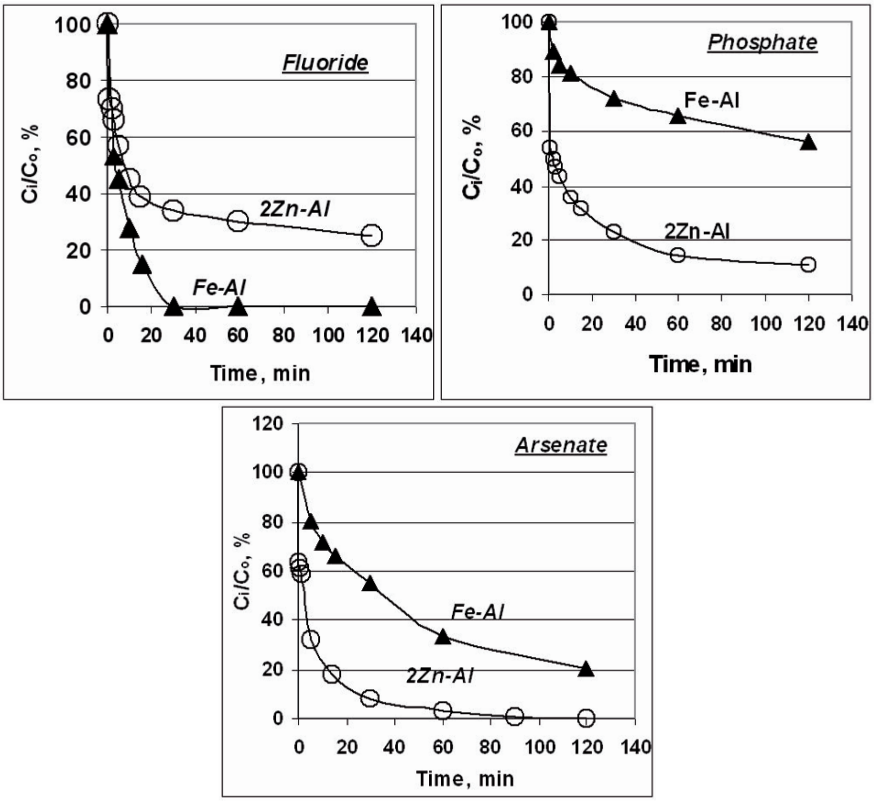
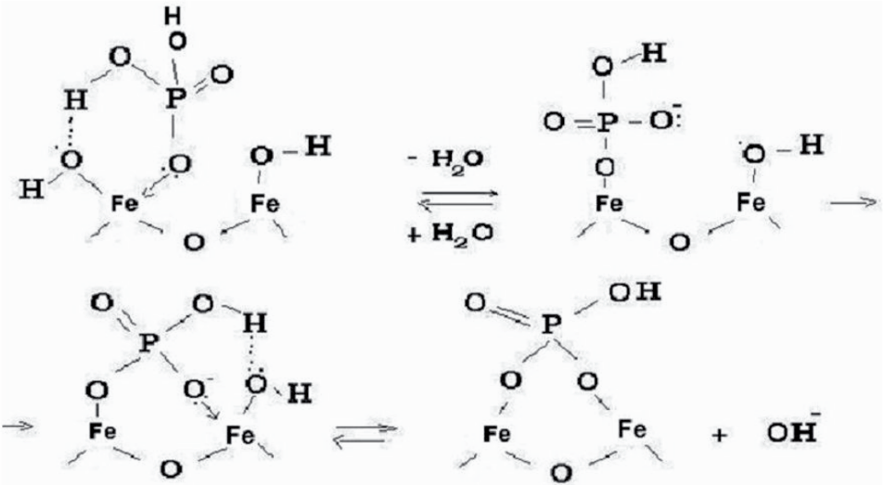


Figure 4. Kinetics of F^-, $H_2PO_4^-$ and $H_2AsO_4^-$ sorption onto Fe-Al and 2Al-Zn. Conditions of the experiment: the initial concentration of the anions was 0.3 mM/L, solids concentration was 2g/L, temperature was 22 ± 2 ºC.

It is also the result of the nucleophilic S_N2-i- replacement of OH^- groups of the Fe atoms by phosphates with the formation of intermediate six-number complex according to the following scheme 3:



Hydrogen bonds are created between the hydroxyl of the sorbent and the other proton of the the singly-charged phosphate anion, and this makes easy the replacement resulting in high sorption capacity. As such a bond between hydroxyl of the surface and the last, third proton of the doubly-charged anion HPO_4^{2-}, is not possible, a weak uptake of this species is observed. So, at pH values 4 to 5 where the $H_2PO_4^-$ predominates, good sorption is observed, while at pH values over 7 where the double charged species (HPO_4^{2-}) predominates, sorption is decreased. The same mechanism is also expected for the other oxy-anions (i.e. chromate, bichromate and arsenate) and a high capacity of hydrated oxides of Al, Fe, Zr and Ti toward these anions is expected. It is proposed that the effectiveness of this nucleophilic replacement decreases as following: Zr>Al>Fe>Ti in accordance with their acceptor ability.

Suggestions about the mechanism were also drawn from some modeling of the adsorbent (4Fe-4Al) structure with HyperChem7 quantum chemistry program. Figure 5 shows the model cluster of the synthesized sorbent containing an equal number of iron and aluminium atoms. Electronic structures of this cluster (geometry and charge distribution) have been calculated using PM3 method. In this model, the aluminium atom clusters with three other atoms, while the iron atom has octahedral and tetrahedral configurations. Oxygen is two- and three-coordinated here. The charges on atoms of aluminium, iron, and also oxygen of hydroxyl groups linked with aluminium and iron have been shown. There is a large positive charge (near + 1 e) onto the aluminium atom, but small negative charge is on iron atoms. This indicates that electronic density has been transferred from oxygen atoms linked with iron.

Analysis of the local functions (on atoms) of the density of states (DOS) (Figure 6) has shown that the atoms of oxygen and iron have considerable electron-donor properties, while atoms of aluminium and oxygen have electron-acceptor properties. It is worthwhile to notice that the octahedral configuration of iron atoms has more electron-acceptor properties but the tetrahedral configuration has more electron-donor properties. At that, three-coordinated atoms of oxygen have the highest electron-donor and electron-acceptor properties. Oxygen atoms of hydroxyl groups are not responsible for electron-acceptor properties.

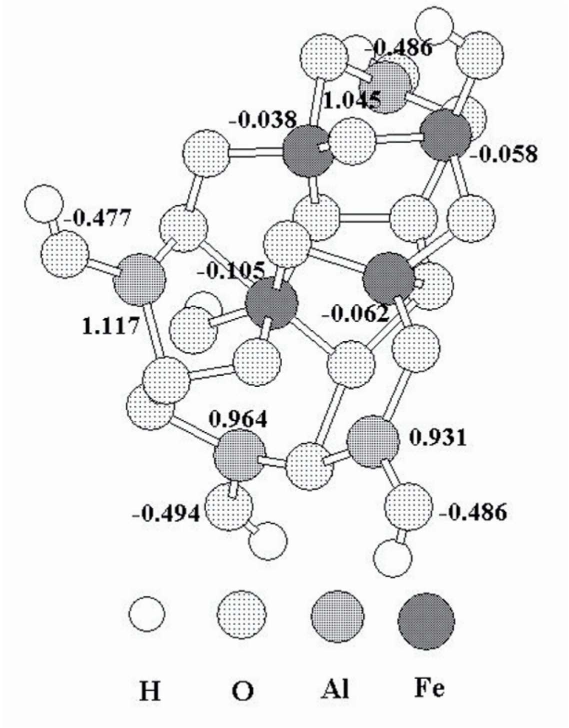


Figure 5. Scheme of the model cluster of 4Fe-4Al.

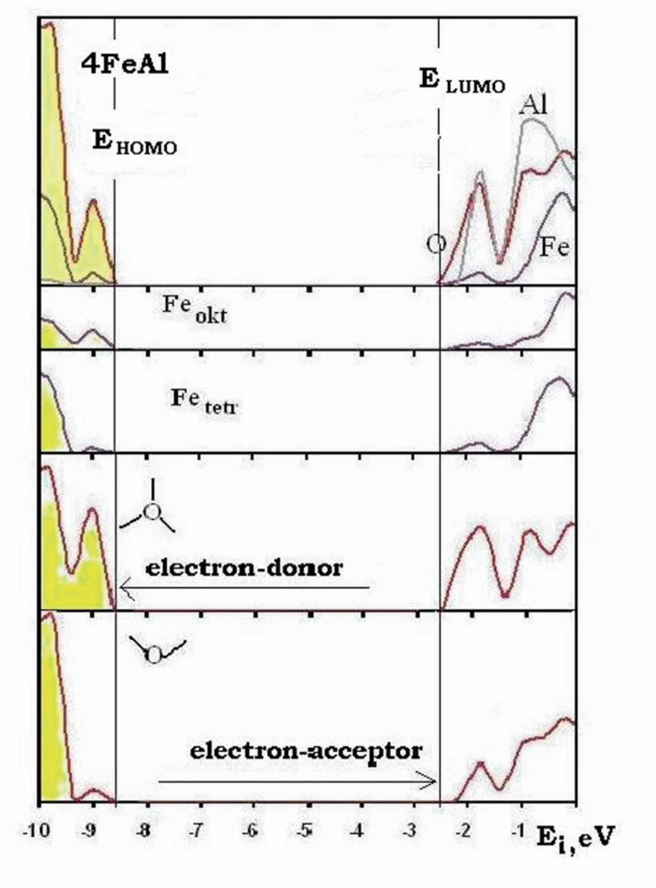


Figure 6. Local functions of the density of states (DOS) of cluster 4Fe-4Al.

Charge distribution on the surface, shown in Figure 5, indicates that considerable positive charges, concentrated on aluminium, are the most probable (reasonable) coordination sites for negatively charged halides. The mechanism of this reaction has been demonstrated by interaction of NaF with the investigated model cluster (Figure 7). Taking into account that dipole moments of the cluster and NaF are correspondingly 7.07 D and 8.11 D, thus electrostatic interaction, which causes orientation and approaching the molecule NaF to atom of Al having the biggest charge (q=1.117 e) on the cluster, will be dominating at considerable distances between NaF and cluster (1, 2 and 3 on the Figure 7). Optimization of the geometry of the cluster (3, Figure 7) by the PM3 method allows concluding that during interaction the transition reactive complex with four-coordinated Al (AlO_3F) has been formed. Such interaction leads to redistribution of the charge density, separation of Na^+ ion, formation of hydrated cation of Na^+, which interacts with OH^- of four-coordinated Al forming NaOH (5, 6, Figure 7). Isolation of OH^- and alkalization of the experimental solution is also confirmed by the experimental data. A final result of this interaction is a cluster with bound fluoride. It is worth to notice that gain of heat formation of F-containing cluster as compare with the initial cluster is 37 kcal/M.

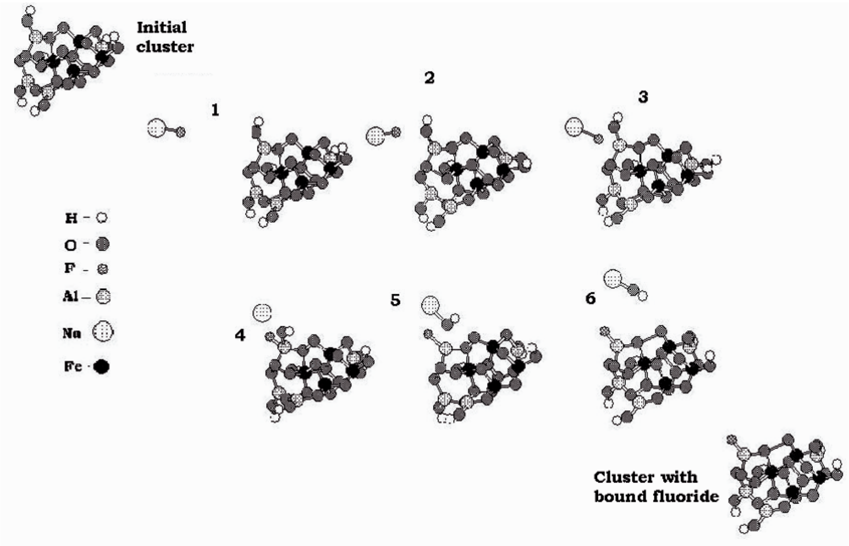


Figure 7. Mechanism of fluoride binding to the surface of 4Fe-4Al.

4. CONCLUSIONS

The new ion exchangers based on mixed hydrous oxides of Fe, Al and Zn of general formula $Fe_2O_3{\cdot}Al_2O_3{\cdot}xH_2O$ and $2Al_2O_3{\cdot}ZnO{\cdot}xH_2O$ were obtained by the original sol-gel method. This technique allows running sol-gel process using cheap raw materials (simple salts, alkali, acids).

In accordance to potentiometric titration data, the anion exchange capacity of $2Al_2O_3{\cdot}ZnO{\cdot}xH_2O$ was almost twice higher than that of $Fe_2O_3{\cdot}Al_2O_3{\cdot}xH_2O$ in spite of the fact that specific surface area of the latter one was 396 m^2/g against 33 m^2/g for $2Al_2O_3{\cdot}ZnO{\cdot}xH_2O$.

The two new ion exchangers were employed for adsorption studies of phosphate, arsenate, fluoride and humic acids.

The technologically important pH effect was found for both materials. They can be used for removal of the investigated anions at the wide range of pH from 3 to 10. At that, maximal sorption of all investigated anions was in acidic pH what is typical for sorption of anions.

Equilibrium sorption isotherms of all investigated anions obtained at pH 7 onto both ion exchangers have shown that $Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$ has much higher sorption capacity and affinity toward F^- than does $2Al_2O_3 \cdot ZnO \cdot xH_2O$. Tetrahedral anions ($H_2PO_4^-$ and $H_2AsO_4^-$), on the contrary, were much better adsorbed to the surface of layered $2Al_2O_3 \cdot ZnO \cdot xH_2O$ showing exceptionally high for the moment sorption capacity toward As(V) at this pH (140 mg/g).

Kinetics data were in good agreement with isotherms. The rate of F^- adsorption onto $Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$ was much higher than onto $2Al_2O_3 \cdot ZnO \cdot xH_2O$, and on the contrary, rate of adsorption of $H_2PO_4^-$ and $H_2AsO_4^-$ was much faster than onto $Fe_2O_3 \cdot Al_2O_3 \cdot xH_2O$.

Quantum-chemistry modeling with HyperChem7 was applied for such systems, what allowed modeling of the cluster of the adsorbents and to assume the mechanism of sorption.

The data shown in this paper allow for the consideration of the new adsorbents as promising for their potential application at drinking water treatment to solve the task of the selective removal of anions from purifying waters.

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XENOBIOTIC PHARMACEUTICALS IN WATER AND METHODS TO PREVENT THEIR APPEARANCE IN DRINKING WATER

Photolytic and Photocatalytic Degradation of Pharmaceuticals

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Abstract: The aim of this work was to investigate the sun light induced photodegradation as well as the photocatalytic degradation of pharmaceuticals and X-ray diagnostic agents (PhaDAs), and to follow their degradation pathways. The influence on the photolytic and photocatalytic degradation of the selected PhaDAs in the absence and presence of natural organic matter was investigated. Technical systems suitable for photocatalytic degradation and adsorption were developed and their applicability was checked. The advanced oxidation processes and adsorption experiments led to promising data for the elimination of the PhaDAs.

Key words: solar photochemistry; simulated sun light; natural elimination; heterogeneous photocatalysis; pilot plant; natural organic matter; adsorption; water treatment techniques

1. INTRODUCTION

In our days, a good part of the population relies on regular medication. In special cases chemotherapy is applied. This has increased the number and amounts of pharmaceuticals and diagnostic agents (PhaDAs) produced. Some have turned out to be poorly biodegradable and very soluble in water. This led to a ubiquitous appearance in the aquatic environment via waste water.

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Soil and Water Pollution Monitoring, Protection and Remediation, 3–23.

After application, a good deal of the PhaDAs find their way via excretion, sewage and waste water treatment plants to aquatic ecosystems. In an uncontrolled way unused drugs can also find their way via waste and landfill sites into the groundwater. Similar pathways also exist for the veterinary drugs and food additives in farming which reach the environment as components of liquid manure (Ternes, 1998; Halling-Sørensen et al., 1998).

From the broad variety of compounds we selected clofibric acid, the active metabolite of lipid lowering agents, carbamazepine, an antiepileptic and analgetic product, and iomeprol as nonionic triiodinated X-ray contrast media. The applied masses of these compounds in Germany and in England are estimated to be in the range of 10 to 100 t/a each (Stumpf et al., 1996; Stan and Heberer, 1997; Jones et al., 2002). About 3500 t/a of the family of the triiodinated X-ray contrast media are applied worldwide (Putschew et al., 2000). This has led to typical concentrations of up to several µg/L per compound in sewage effluents and to ng/L concentrations in surface water (Daughton and Ternes, 1999; Heberer, 2002). In Lake Constance (Bodensee), Germany, which is used as resource for drinking water, about 10 to 40 ng/L of each compound has been found in different water depths down to 250 m (Roßknecht et al., 2001).

2. DRINKING WATER SUPPLY

In Central Europe, drinking water supply normally relies on the Multi Barrier System. That means that several steps are considered for the sake of precaution for human health. They include e.g. state of the art of technical wastewater treatment, protection of aquatic systems including surface and groundwater, selection of the best resource available for raw water, bank filtration, state of the art of drinking water treatment plants, critical control of disinfection capacities, effective monitoring systems for water quality, reliable construction and management of distribution systems.

From the recently obtained data it is clear that despite of all the common precaution measures there are obvious leaks for some of the PhaDAs in the water cycle. Their poor biodegradability and weak adsorbability as well as their fairly high water solubility are the main reasons for their persistent behavior and wide distribution. Biological waste water treatment can only remove 30 to 50% of the clofibric acid present in the effluent, less than 10% of the carbamazepine and practically nothing of iomeprol (Ternes, 1998; Stumpf et al., 1999; Ternes and Hirsch, 2000; Clara et al., 2004).

In drinking water treatment, clofibric acid, carbamazepine and the iodinated compounds can partly be removed by bank filtration (Putschew et al., 2000; Brauch et al., 2000) and activated carbon (Sacher et al., 2001; Ternes et al., 2002). Flocculation / coagulation and ozonation have turned out to be fairly ineffective for removal (Ternes et al., 2002; Huber et al., 2003; Ternes et al., 2003). Information on the efficiency of advanced oxidation processes (AOP) like heterogeneous photocatalysis and on photochemical degradation, however, is not available so far. Therefore we investigated these approaches for the degradation of pharmaceuticals in water and followed the degradation pathways. Application of advanced oxidation processes and adsorption experiments led to promising data for the elimination of the PhaDAs.

The aim of this chapter is to:

1. discuss the basics for sunlight induced as well as photocatalytic degradation,
2. show results from experiments for the photolytic and photocatalytic degradation of the selected PhaDAs in the absence and presence of natural organic matter (NOM),
3. conclude on the development of technical systems suitable for photocatalytic degradation and
4. discuss the subject specific aspects of a sustainable development of aquatic systems with respect to the obvious dilemma between health care and environmental protection.

3. EXPERIMENTAL DETAILS

Iomeprol was a courtesy gift from Altana, clofibric acid and carbamazepine were purchased from Sigma-Aldrich. P25 (ca. 25% rutile and ca. 75% anatase) was purchased from Degussa, and Hombikat UV100 (100% anatase) was a courtesy gift from Sachtleben Chemie. The characteristics of the different TiO_2 powders are given elsewhere (Doll and Frimmel, 2004). NOM was taken from Lake Hohloh (code HO19), a brown water lake in the Black Forest (Germany), which has a high concentration of DOC (ρ_0(DOC) = 21.1 mg/L). Subsequently, the sample was filtered (0.45 µm, cellulose nitrate) and stored in the dark at 4 °C. The measured basic characteristic data of the nondiluted bog water HO19 are given elsewhere (Doll and Frimmel, 2003).

The samples were irradiated using a sun light simulator (Oriel Corp.) with additional filters installed in the radiation beam to match the spectrum of the lamp to the natural sun light spectrum (Figure 1). The radiation source was a 1000-W Xe short-arc lamp. Up to 9 samples were irradiated simultaneously from above in a homogeneous light field. The optical pathlength of each sample was 1.7 cm with a surface area of 12.1 cm^2. The samples were cooled (20 °C ± 2 °C) by circulating water in a bath and stirred magnetically. The incident photon flux was determined by chemical polychromatic actinometry, using phenylglyoxylic acid (Defoin et al., 1986).

Analyses of clofibric acid, carbamazepine, iomeprol and their degradation products were carried out by HPLC (HP1090) using a Hypersil ODS (Agilent) column with diode-array and fluorescence detection. The separation of I^- and iomeprol was performed by HPLC (Sykam S1121) using an IonPac®AS9-SC (Dionex) analytical column. Iodine was detected on-line with ICP-AES (Vista-Pro CCD Simultaneous ICP-OES, Varian). The operating conditions for the HPLC measurements, the description of DOC (dissolved organic carbon) and AOI (on activated carbon absorbable organic iodinated compounds) measurements are given elsewhere (Doll and Frimmel, 2003).

Stock solutions were prepared by dissolving the pharmaceuticals and contrast media in Milli-Q purified water (Millipore). After addition of the pharmaceuticals, the samples were stirred for several hours to ensure complete dissolution and filtered (0.2 µm cellulose nitrate filter). The stock solutions were protected from solar irradiation and stored in a refrigerator. They were kept less than one month. For experiments the model solutions containing NOM, HO19 and the stock solution of PaDAs were mixed and diluted to get the final concentrations. For the irradiation experiments the TiO_2 suspension was freshly prepared by suspending TiO_2 in Milli-Q purified water followed by sonication for at least 30 min. Before starting the irradiation (photolytic and photocatalytic), the pharmaceutical or contrast media solution was equilibrated with humidified air in order to get defined concentrations of oxygen and bicarbonate in all samples, then mixed with the sonicated TiO_2 suspension (photocatalytic) and adjusted to a defined pH value. After photocatalytic irradiation, the samples were filtered through a membrane (PVDF, Millipore) with a pore size of 0.1 µm or centrifuged with an ultracentrifuge (Optima TLX, Beckmann Coulter) for the DOC measurements.

Adsorption experiments were done in bottles by shaking the samples overhead for 48 hours protected from light with variation of the sorbate (iomeprol: 0.4 mg/L up to 25 mg/L) at constant TiO_2 concentration (1 g/L). The initial pH value (pH: 6.5) and the sample treatment before and after the adsorption time was analogous to the irradiation experiments.

4. RESULTS AND DISCUSSION

4.1 Spectral Characterization

Sunlight on the earth's surface shows a spectrum from 300 nm up to longer wavelengths (ca. 800 nm). Especially the range between 300 and 400 nm (UV-A) is under investigation with regard to climate change effects. The sun light simulator in the reported experiments showed a spectrum which was in this range in good agreement with natural sunlight of a summer day.

The spectral irradiance of the sun in January and June in Central Europe according to Frank and Klöpffer (1988) and of the sun light simulator according to Schindelin and Frimmel (2000) are given in Figure 1. The comparison of the photon flux (Doll and Frimmel, 2003) showed that the sun light simulator depending on season was 1.3 to 20 times higher than the real sun light.

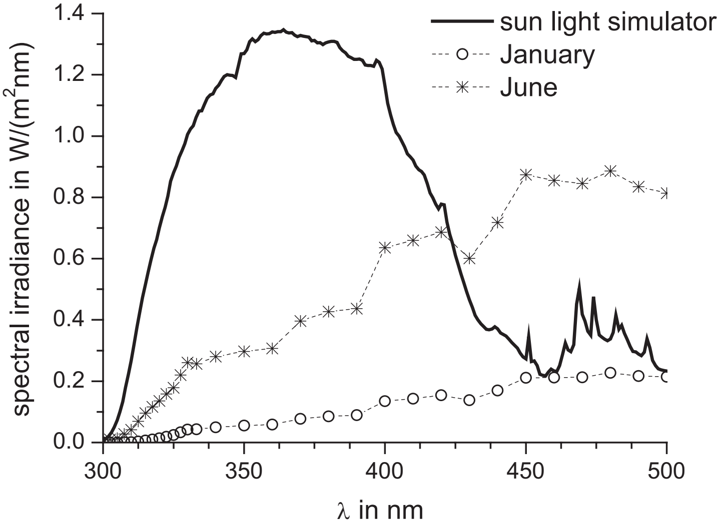

Figure 1. Radiation of the sun light simulator and the sun (January and June).

Photochemical reactions start with radiation absorption. Therefore the UV/Vis spectra of the substances concerned are of basic importance. Figure 2 shows the spectra of aqueous solutions of the investigated PhaDAs and of NOM which is ubiquitous in all surface waters. It is obvious that mainly the short wavelength of sun light gets absorbed by the substances.

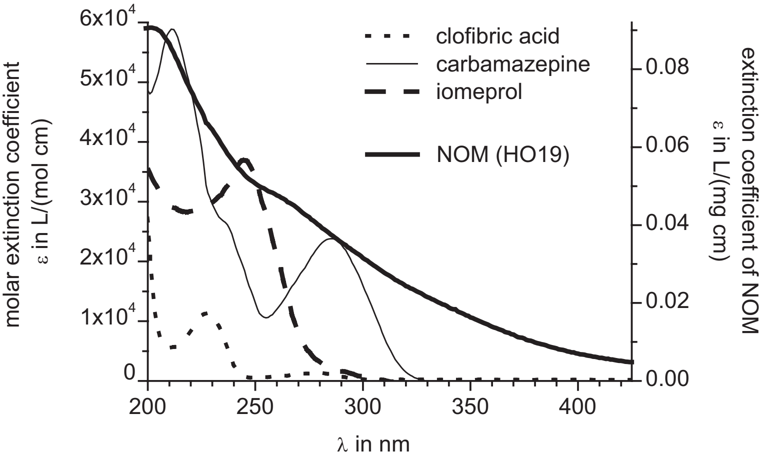

Figure 2. Spectral absorbances of the drugs and NOM.

4.2 Photodegradation

The photodegradation of iomeprol by irradiation with the sunlight simulator is shown in Figure 3. It degrades with irradiation time. The decrease of AOI is somewhat less than which can be explained by partial degradation to organic products with bound iodine. Release of iodine is shown in the circles of iodide (Doll and Frimmel, 2003).

In the presence of NOM, the photochemical reactions are more complicated. It is known that NOM plays an important role as inner filter, shielding other molecules from radiation. This has to be considered especially for the photic zones of surface waters. On the other hand, irradiation of NOM can also lead to radical formation. In case O_2 is present, several reactive species including OH-radicals are formed (Zepp et al., 1985; Frimmel, 1994; Frimmel, 1998). Parts of them get scavenged by NOM and by this they can react. As a consequence, the different pathways have to be kept in mind and contribute to the all overall yield of degradation. The degradation of carbamazepine in the presence of different NOM concentrations (given as DOC) is shown in Figure 4 and the data can be used to calculate the degradation rate according to first order (Doll and Frimmel, 2003).

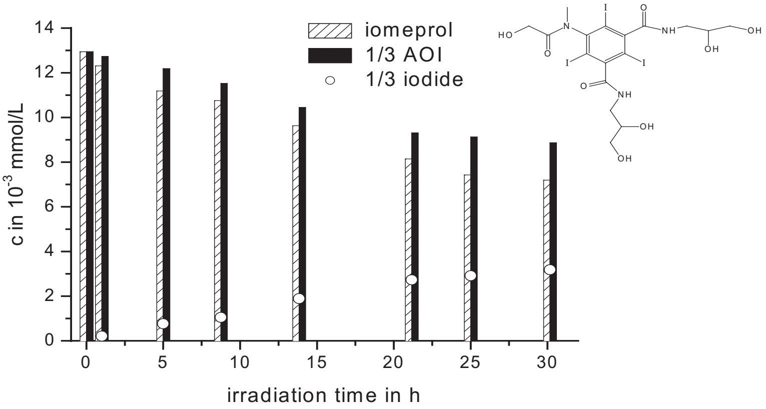

Figure 3. Photodegradation of iomeprol.

The inner filter effect of NOM quantified in Doll and Frimmel (2003) showed no significant contribution to the experimentally determined rate constants for the photodegradation of carbamazepine. Obviously the NOM stimulated the formation of reactive radical species which is the dominating influence at different NOM concentrations (ρ(DOC) < 20 mg/L) (Doll and Frimmel, 2003).

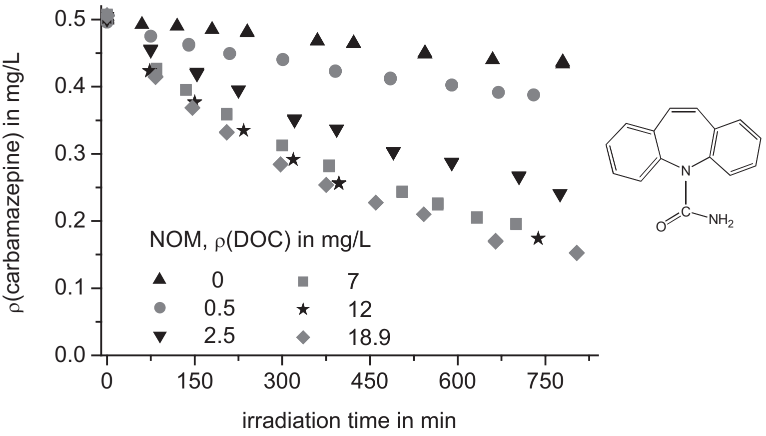

Figure 4. Photodegradation of carbamazepine in the presence of NOM.

4.3 Photocatalytic Degradation

Based on the photodegradability of PhaDAs under conditions of the photic zones of surface waters, it is attractive to ask for the degradability of these compounds by photocatalysis. It might lead to much faster rates and could therefore be successfully applied in wastewater treatment, especially in regions with intensive sun light. An attractive photocatalyst is TiO_2 since it is easily available, economically reasonable and its basic function is quite well understood (Hoffmann et al., 1995; Linsebigler et al., 1995). The results for heterogeneous photocatalysis obtained so far suggest that the considered molecules need to be adsorbed on the catalyst before they get degraded (Doll and Frimmel 2005a). Therefore adsorption isotherms for the PhaDAs on the TiO_2 modifications were determined according to the Freundlich isotherm. For iomeprol the Freundlich constant K_F of the Hombikat UV100 and P25 was 0.767 mg/g $(L/mg)^n$ and 0.196 mg/g $(L/mg)^n$, respectively. K_F for Hombikat UV100 was about 4 times higher than that of P25, which clearly reveals the higher adsorption capacity of Hombikat UV100. This goes along with a better photocatalytic degradation of iomeprol by Hombikat UV100 in the absence and presence of NOM or iopromide (Doll and Frimmel, 2005b), e.g. for iomeprol (ρ_0 = 5 mg/L) the photocatalytic degradation rate constant with the TiO_2 (ρ = 0.5 g/L) Hombikat UV100 and P25 was 0.0093 1/s and 0.0053 1/s, respectively. The photocatalytic degradation of iomeprol was controlled by the analysis of iomeprol, DOC, AOI and I^-. Again it was obvious that the degradation includes the formation of several quite stable organic and iodinated products (Doll and Frimmel, 2004). Their identification is under investigation.

The photocatalytic degradation of clofibric acid (ρ_0 = 200 mg/L) using P25 (ρ = 0.5 g/L) (Figure 5, according to Doll and Frimmel (2004)) also shows clearly the formation of several intermediate products. The DOC balance points out that in addition to the identified products some other ones are still missing.

In contrast to the photolysis of carbamazepine (Figure 4) its photocatalytic degradation on P25 (ρ = 0.1 g/L) is clearly inhibited by NOM as shown in Figure 6, according to Doll and Frimmel (2005b). It is attractive to assume the blockage of photocatalytic active sites of TiO_2 by NOM. In addition, a stabilization effect as a result of complex formation between carbamazepine and NOM can be hypothesized and is under further investigation.

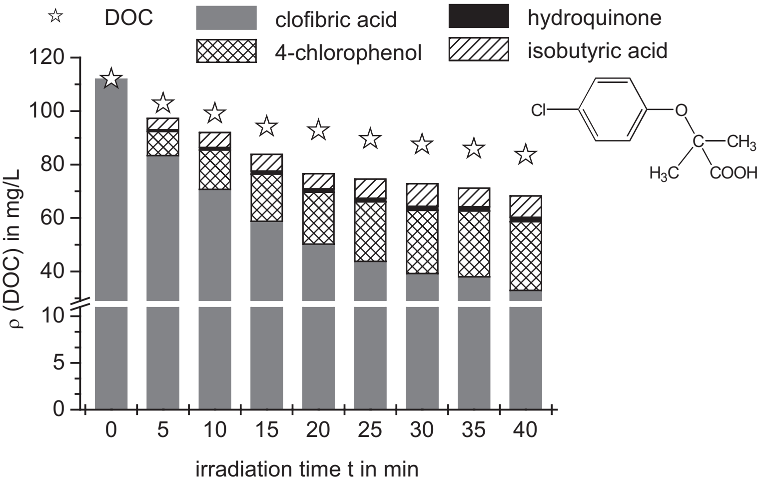

Figure 5. Photocatalytic degradation of clofibric acid.

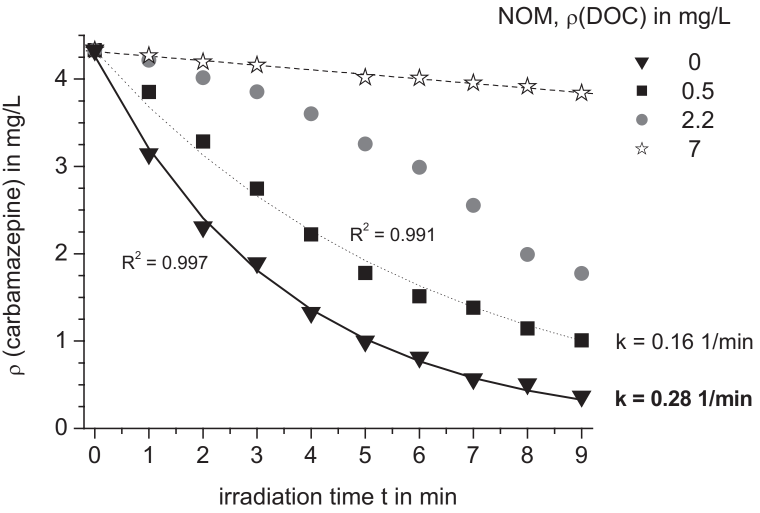

Figure 6. Photocatalytic degradation of carbamazepine in the presence of NOM.

4.4 Technical Applicability

Photocatalysis has proved to be a suitable approach for the elimination of PhaDAs in polluted waters. The application of a photocatalyst in a heterogeneous system needs some technical considerations like immobilization of TiO_2 on reactor walls or separation by filtration. A pilot plant has been developed which combines an adsorptive step of the PhaDAs on TiO_2, a separation step of the loaded catalyst in a membrane module and an irradiation step for regeneration of the TiO_2 in the retentate of the membrane separator. The permeate consists of the cleaned water or product, and the TiO_2 can be recycled in that procedure (Figure 7, according to Doll and Frimmel (2005c)).

A crucial point in recycling TiO_2 is its long time activity and stability. So, the continuous degradation of iomeprol, the mixture of clofibric acid and carbamazepine over several days was investigated (Doll and Frimmel, 2005c). Obviously, there was no loss in the photocatalytic activity of the catalyst over the whole period of operation.

Having this in mind, photocatalysis e.g. with TiO_2 seems to be a promising method for PhaDAs removal with broad application in cleaning process water, waste water from hospitals, old people's homes and municipalities, and even drinking water treatment. The low consumption of chemicals and energy makes the method a good example of green chemistry and modern technology.

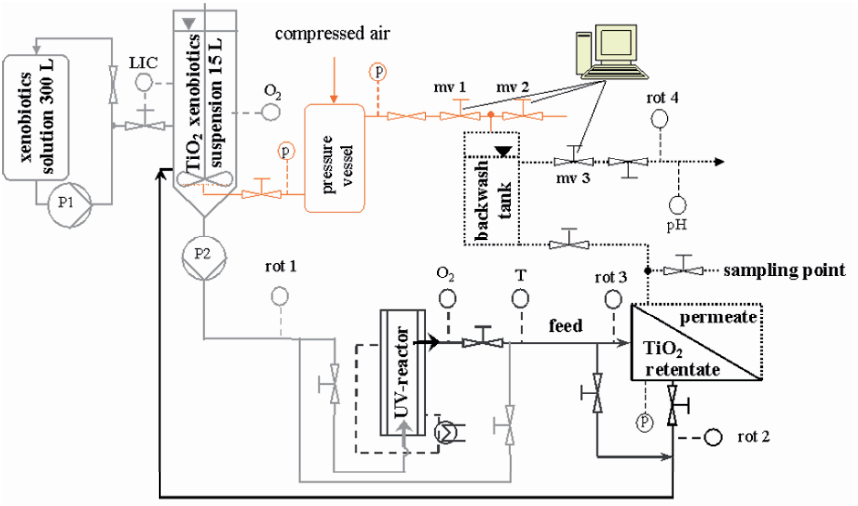

Figure 7. Pilot plant for combined catalytic oxidation and microfiltration.

5. SUSTAINABILITY

Photolytic and photocatalytic degradation turned out to be a promising tool for a continuous process for the degradation of PhaDAs. The possibility of regeneration and recycling of the photocatalyst is promising.

It is reasonable to see all our activities in the context of sustainability, i.e. in caring about the next generations. This also brings the PhaDAs as necessities for health into the focus of a clean environment and especially well functioning aquatic ecosystems as basis for life. Production integrated water protection with an increasing amount of recycled process water is the technical necessity of our days. In addition, the product integrated water protection asks urgently for the usage of natural products or the design of ecologically safe xenobiotics which nature can handle easily. Finally, the consumer integrated water protection has to be reinforced by education and truthful information. The individual power in selecting green products and clean approaches towards the aquatic systems have been underestimated and deserve full support since it is vital for the survival of mankind.

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UV/VIS LIGHT-ENHANCED PHOTOCATALYSIS FOR WATER TREATMENT AND PROTECTION

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Abstract: Applied research data on advanced photocatalytic methods aimed at treatment of contaminated aqueous phase is presented. Phenol and chloroorganic pesticides (as model compounds) besides actual wastewater samples were UV-photodegraded using suspended TiO_2 or UV/H_2O_2/air systems. Titanium dioxide catalysts modified with non-metal elements were used to photodegrade phenols in visible light. Laboratory and pilot scale reactors were used and the effect of process scale-up investigated.

Key words: organic pollutants; photodegradation; water treatment; titanium dioxide; gas-sparged reactor

1. INTRODUCTION

Advanced methods are in demand for removal of persistent organic pollutants from waste waters and ground waters. Semiconductor-assisted photocatalysis has been found supplementary and complementary to conventional approaches aimed at destruction or transformation of hazardous chemical waste such as high temperature incineration, activated sludge digestion, anaerobic digestion, and physicochemical treatment (Hoffmann et al., 1995; Doll and Frimmel, 2005).

Groundwater contamination is likely to be the primary source of human contact with toxic chemicals originating from dump site leachates, but also from wastewater discharges to ground and surface waters. Of concern are

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solvents, volatile organics, chlorinated volatile organics, dioxins, dibenzofurans, pesticides, PCB's, chlorophenols, drugs, dyes, heavy metals and arsenic compounds.

Photochemical oxidation processes, based on the generation of highly reactive hydroxyl radicals ($^{\bullet}OH$) using UV/TiO_2 and UV/H_2O_2 methods, can lead to degradation or mineralization of most contaminants.

Photocatalytical degradation is attractive for treatment of waste streams which are too dilute for incineration and too concentrated for biological treatment or contain highly toxic organic compounds. Although a number of possible degradation pathways can be envisioned, the formation and subsequent reactions of hydroxyl radicals, being a very strong oxidizing agent (standard redox potential +2.8 V), are generally accepted as predominant degradation pathways of organic substrates in oxygenated aqueous solutions (Topalov et al., 2001).

Titanium dioxide (TiO_2) has been known as a semiconductor exhibiting substantial photocatalytic activity. It is non-toxic and stable in aqueous solutions (Hsien et al., 2000) and relatively inexpensive. When radiation of energy equal to or greater than the bandwidth of the semiconductor is absorbed, (see Figure 1) a photon excites an electron from the valence band to the conduction band and leaves an electronic vacancy (a hole) in the valence band (h^+). The photo-generated holes at the surface of the irradiated semiconductor can oxidize a variety of hazardous species directly to water or produce $^{\cdot}OH$ radicals.

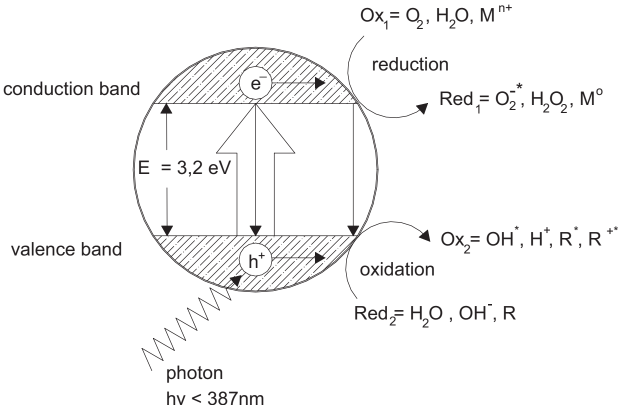

Figure 1. Mechanism of UV/TiO_2 photocatalysis (Hoffmann et al., 1995).

Catalyst powder can adhere to the UV lamp and reactor wall. To enhance its separation various supports have been used: molecular sieves (Hsien et al., 2001), steel plates (Brigden et al., 2001), glass plates (Macounová et al., 2001) and silica. In our work glass microspheres recovered from fly ash were tested as the catalyst carrier.

Practical application of photocatalysis is limited by the fact that TiO_2 absorbs only UV light due to its high bandgap energy. Only 2 to 5% of the solar spectrum (UV fraction of solar light) can therefore be utilized by conventional TiO_2-photocatalysis. Thus, UV lamps are needed when TiO_2 is used, which leads to an increase of operating costs of the UV/TiO_2 system. Visible light-induced reactions on TiO_2 can be obtained by sensitization of titania surface (indirect mechanism) or by modification of its structure to shift absorption spectrum to visible light region (direct mechanism of photocatalysis). Modification of the chemical structure involves introduction of metals (Anpo, 2000) (Cr, Pt, V, Nb, Fe, Ru) and non-metals (Asahi et al., 2001; Sakthivel and Kisch, 2003; Umebayashi et al., 2002) (N, C, S, B) or transformation of TiO_2 to a non-stoichiometric form (Justicia et al., 2002). In our investigations modified TiO_2 catalysts active in visible light were prepared.

2. EXPERIMENTAL SYSTEMS

2.1 Mercury Lamp – Based Reactor

Model pollutants dissolved/dispersed in water were photodegraded in a water-cooled laboratory UV reactor /2/, equipped with a Heraeus 150 W medium-pressure mercury vapour lamp /1/, see Figure 2. An appropriate amount of powdered titanium dioxide (as anatase or rutile) or microspheres coated with TiO_2, was suspended in 800 ml of an aqueous solution. The aqueous titanium dioxide suspension was stirred for 10 min. and then irradiated in the presence of air, oxygen or argon, sparged through the solution at a rate of 10 or 20 dm^3/h. The gas phase leaving the reaction environment was passed through an absorption solution /7/ to trap volatile organic compounds. The pH and the concentration of Cl^- anions were monitored *on-line* using pH and ion-selective electrodes. Data were collected each minute and the retention time was approximately 3 min.

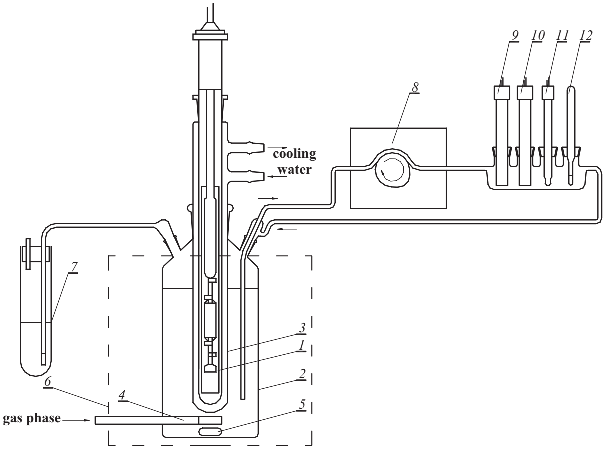

Figure 2. Experimental set-up used for degradation of impurities in aqueous solutions: 1 - Heraeus medium pressure mercury lamp, 2 - glass reactor, 3 - UV lamp cooling system, 4 - sparged gas system, 5 - magnetic stirrer, 6 - thermostat, 7 - absorber, 8 - peristaltic pump, 9 - ion-selective electrode, 10 - reference electrode, 11 - pH electrode, 12 – thermometer.

2.2 Xenon Lamp System

The photocatalytic activity of catalysts under visible light was estimated by measuring the decomposition rate of phenol or 4-chlorophenol in an aqueous solution or acetaldehyde in gaseous phase. The schematic diagram of the experimental set-up for photocatalyst activity testing is presented in Figure 3. The irradiation source was a 1000 W or 150 W Xenon lamp.

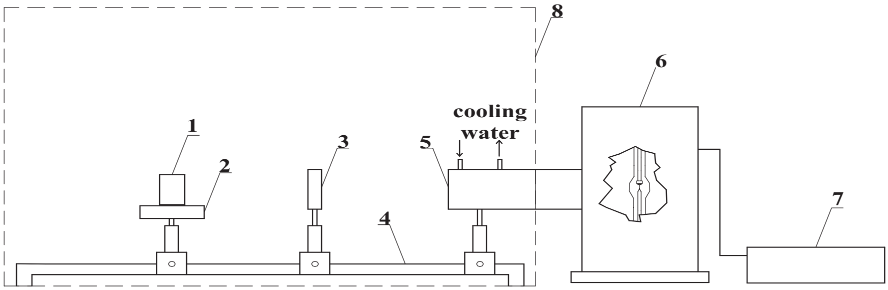

Figure 3. Schematic diagram of experimental set-up for photocatalyst activity testing: 1 - quartz cuvette, 2 - magnetic stirrer, 3 - cut-off filter, 4 - optical train, 5 - water filter, 6 - Xenon lamp, 7 - power supply, 8 - black-box.

The optical path included water and glass filters to cut off IR irradiation and wavelengths shorter than 400 nm or 455 nm, respectively. A magnetic stirrer was used to maintain 25 cm^3 of catalyst (125 mg or 500 mg) in suspension during the aeration (5 dm^3/h) prior to photolysis and during the photocatalytic process. Aliquots of 1.0 cm^3 of the aqueous suspension were collected at regular time periods during irradiation and filtered through syringe filters (Ø=0.2 μm) to remove catalyst particles. The phenol concentration was estimated by a colorimetric method using a UV-VIS spectrophotometer (DU-7, Beckman).

2.3 Gas-Sparged Hydrocyclone Reactor

A thin layer of intensely aerated liquid phase is formed in the air sparged hydrocyclone reactor (ASH), already used as oil flotator (Miller and Hupka, 1983; Miller et al., 1993; Beeby and Nicol, 1993), stripper (Lelinski and Miller, 1996) and aerator (Bokotko and Hupka, 1996). In our research, the ASH for the first time was used as contactor for photocatalytic reactions. Model wastewater containing phenol, chloroorganic pesticides (lindane, DDT, DMDT) and real pesticide wastewater were selected for the tests. Degradation experiments were carried out in the UV, UV/H_2O_2 and UV/TiO_2 systems.

Keeping in mind process scale-up, sufficient light penetration into the solution and availability of oxygen require reactors of special design. Oxygen (air) introduction is important for two reasons: it delivers necessary amount of oxidant and intensifies mixing. In our investigations – a gas sparged reactor was used for carrying out photochemical reactions. The centrifugal force and the shear stress of the swirling liquid in the reactor result in generation of fine gas bubbles below 300 μm (Miller et al., 1985). A thin layer of aerated liquid is well illuminated by swirling around the UV lamp placed in the center of the reactor.

A schematic diagram of the pilot plant scale photoreactor is presented in Figure 4. A gas-sparged reactor /2/ with 1.5 to 4.5 m^3/h nominal flow rate of the aqueous phase, equipped with an axially placed Hanovia 2000 W medium-pressure mercury vapor UV lamp /3/ was used.

Technological runs included photocatalytic degradation of organic contaminats in an aqueous suspension of titanium dioxide that was fixed to the surface of glass microspheres (see Figure 5) (Rzechula et al., 1998). Model wastewater containing phenol, chloroorganic pesticides (lindane, DDT, DMDT) and real pesticide wastewater were degraded in UV, UV/H_2O_2, UV/H_2O_2, UV/TiO_2 systems.

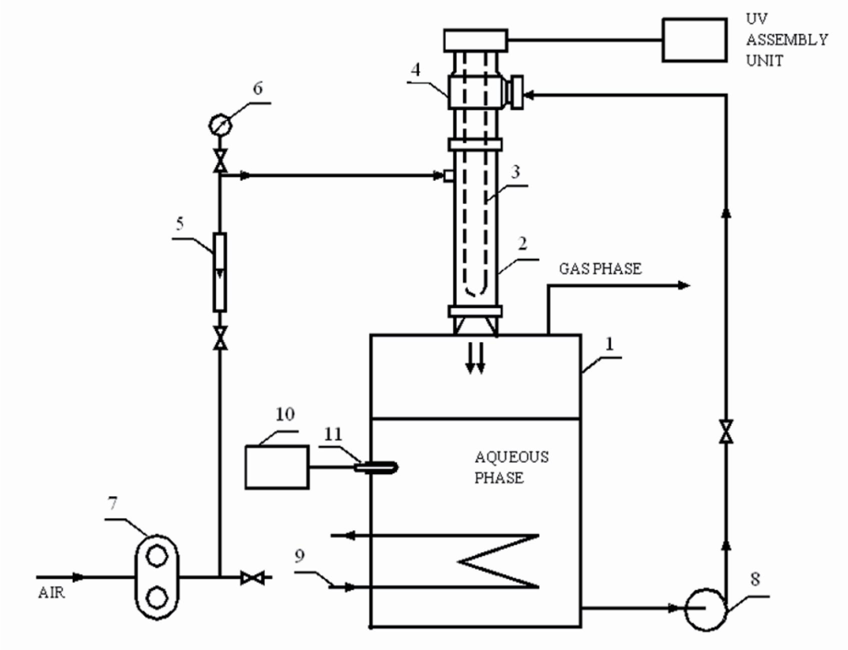

Figure 4. Schematic diagram of gas-sparged reactor system used for photodegradation: 1 - tank, 2 - gas-sparged reactor, 3 - UV lamp, 4 - cyclone header, 5 - rotameter, 6 - manometer, 7 - blower, 8 - centrifugal pump, 9 - cooling coil, 10 - pH meter, 11 - pH electrode.

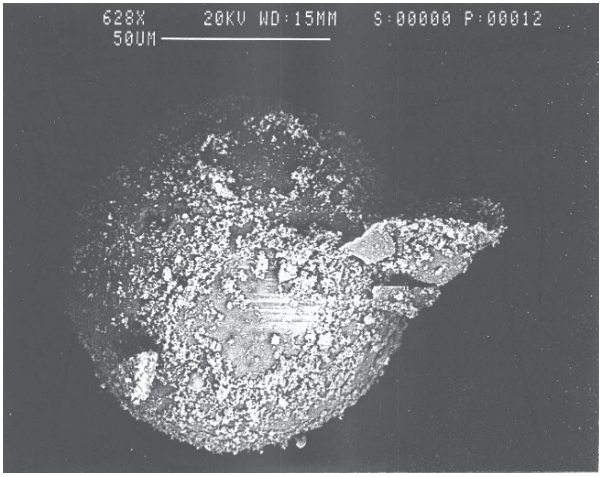

Figure 5. Hollow glass microsphere coated with titanium dioxide.

3. PHOTODEGRADATION RESULTS

3.1 Chloroorganic Pesticides

Lindane, p,p'-DDT and methoxychlor are among the most persistent pesticides in the environment. Although application of some chloroorganic insecticides in agriculture was banned in most countries in the last twenty years, thousands of tons of obsolete pesticide deposits in farms and tombs jeopardize the environment (Zaleska and Hupka, 1999; Amador, 1992, Czaplicki et al., 1996).

The degradation rate of γ-HCH (lindane), p,p'-DDT and methoxychlor and the formation of the α-HCH reaction byproduct were investigated in the presence of powdered anatase, rutile or anatase supported on glass hollow microspheres (Zaleska et al., 2002). More favorable elimination kinetics was obtained for powdered anatase than for powdered rutile for all investigated pesticides. The results suggest that for heterogeneous systems anatase is more photoactive than rutile, which confirms findings from the literature pertaining to other organic compounds. Palmisano et al. (1994) reported that poor photoactivity of rutile resulted from almost irreversible dehydroxylation of the surface and, consequently, due to the negligible capacity for O_2 adsorption.

In the presence of TiO_2 supported on glass microspheres a rapid decrease of lindane concentration was observed at the beginning of the reaction. A 30 min. irradiation eliminated 68% of lindane. A much slower concentration drop was observed during the subsequent 120 min of irradiation. Lindane degradation efficiency in the $TiO_2/UV/O_2$ system using powdered anatase was very close to the efficiency obtained using supported titanium dioxide. Lindane was eliminated in 77% after 150 min. irradiation (Zaleska et al., 2002). Pentachlorocyclohexene and α-HCH were identified after 150 min irradiation in the presence of each catalyst. Trichlorobenzene was detected after irradiation only in the presence of anatase – both powdered and supported (Zaleska et al., 2002).

After 30 min. of irradiation in the presence of TiO_2 deposited on glass microspheres, the remaining p,p-DDT was below 7% of the initial concentration (see Figure 6). The same time of processing with powdered anatase eliminated 70% of p,p'-DDT. After one hour of photodegradation in an anatase aqueous suspension, p,p'-DDT elimination increased to 90% and after two hours to 95%. Analogously to lindane elimination, the lowest efficiency with respect to p,p-DDT was obtained for rutile as the catalyst. Only 50% of p,p'-DDT disappeared after 30 min irradiation, and 85% after 150 min (Zaleska et al., 2002).

Products of DDT dechlorination, such as chlorophenylmethanone, dichlorophenylmethanone, trichloro-benzeno-phenone, DDE and DDD were detected after 150 min. of irradiation. The byproducts were detected after photodegradation of mixture of the three investigated pesticides in an aqueous phase. The degradation products of p,p'-DDT and methoxychlor could be similar, because of the similar structure of these compounds. The degradation byproducts of lindane, DDT and methoxychlor identified in this investigation were confirmed in experiments of photodegradation of single pesticides. The degradation products of 200 mg/dm^3 of individual pesticides suspended in water were reported in earlier paper (Zaleska et al., 1999).

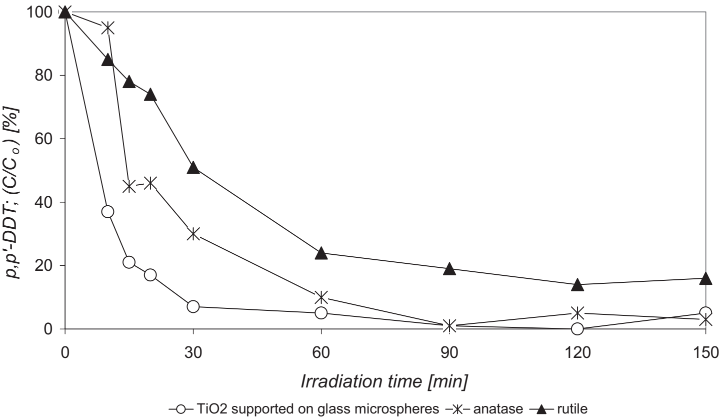

Figure 6. Kinetics of p,p'-DDT photocatalytic degradation in aqueous solution in the presence of powdered TiO_2 (0.5 g/dm^3) and TiO_2 supported on glass microspheres (5 mg/dm^3) at pH 11.

Compared to lindane and p,p'-DDT, methoxychlor exhibited greater susceptibility to photocatalytic degradation (see Figure 7). After 30 min-irradiation over 90% of methoxychlor was degraded in the presence of each investigated catalyst. In the case of titanium dioxide supported on glass microspheres, methoxychlor concentration was less than 10% of the initial concentration after 10 min. irradiation. Only methoxy-benzaldehyde was determined as a degradation product of methoxychlor after 150 min. irradiation. More intermediate compounds were found after 60 min. irradiation of the suspension containing 200 mg/dm^3 of methoxychlor (Palmisano et al., 1994). 1,1'-(Dichloroethenylidene)bis[4-methoxy]-benzene, 1-methoxy-2-[2,2,2-trichloro-1-(4methoxyphenyl) ethyl]-benzene, and 1,1'-(2,2-dichloro-ethylidene)-bis[4-methoxy-]benzene were identified. Apparently these compounds were eliminated after extended processing.

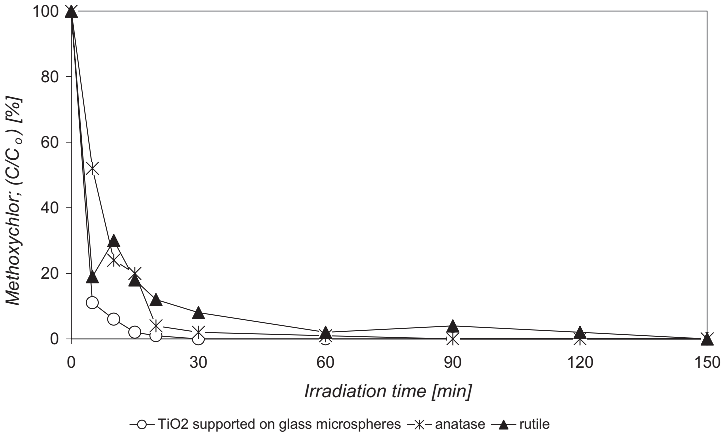

Figure 7. Kinetics of methoxychlor photocatalytic degradation in aqueous solution in the presence of TiO_2 (0.5 g/dm^3) and TiO_2 supported on glass microspheres (5 mg/dm^3) at pH 11.

Photodegradation of lindane p,p'-DDT and methoxychlor in the investigated conditions followed the first order kinetics (Zaleska et al., 2002). The rate constants are provided in Table 1.

Table 1. Rate constants for pesticide photodegradation in the presence of suspended and supported titanium dioxide.

Pesticide	Rate constant k_1 [1/s]		
	TiO_2 supported on glass microspheres	Suspended anatase	Suspended rutile
lindane	0.028	0.033	0.01
p,p'-DDT	0.09	0.032	0.02
methoxychlor	0.3	0.12	0.11

3.2 Phenol - Impact of Oxygen

The experimental data obtained in the TiO_2/UV system (with or without air/oxygen) are presented in Figure 8. For anatase with oxygen sparging the process efficiency reached 72% after 40 min. and was most effective. Oxygen addition resulted in near 40% increase of the reaction efficiency for anatase, 20% for rutile and 10% for microspheres.

The degradation efficiency in the presence of 1.5-7.5 mg/dm^3 TiO_2 supported on glass microspheres was only 20% smaller than that in the presence of 400 mg/dm^3 TiO_2 as powdered anatase. The degradation efficiency in the system without oxygen presence is the same for both titania forms. Anatase occurred to be more photoactive than rutile.

The amount of oxygen affected the photocatalytic reaction efficiency. Oxygen is a source of oxidizing agents, such as: $O_2^{\cdot -}$, $HOO^{\cdot}$, $HO^{\cdot}$ as well as inhibiting charge recombination due to electron capture. A smaller increase of degradation efficiency for anatase supported on glass microspheres after oxygen addition (10%) showed the smaller impact of oxygen. Probably, glass microspheres can scavenge charge carriers (e^- or h^+), thus inhibiting charge recombination. Glass microspheres contain SiO_2 (52-59%), Al_2O_3 (24-31%), Fe_2O_3 (4.6-5.5%), TiO_2 (1-3%). The charge transfer is only possible between excited TiO_2 and Fe_2O_3, see Figure 9.

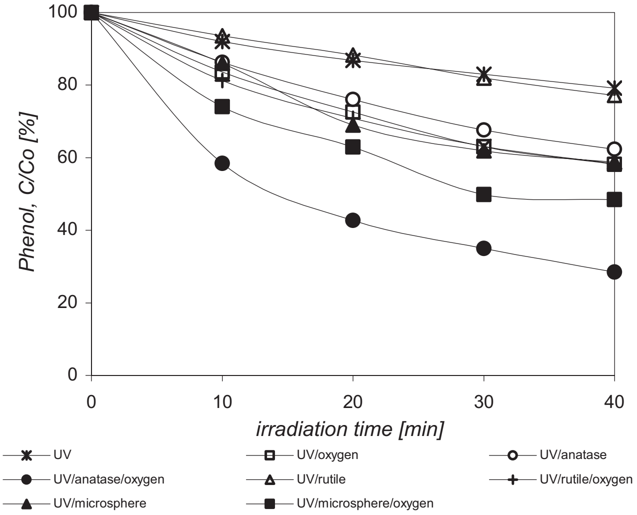

Figure 8. Phenol degradation in the UV/TiO_2 system: Co=60 ppm, TiO_2=0,5 g/dm^3, microspheres - 1 g/dm^3 (15.45 mg TiO_2/dm^3).

3.3 Modification of TiO_2 Towards Activity in Visible Light

To prepare titanium dioxide-based photocatalysts active in visible light, non-metalic precursors containing nitrogen and/or sulphur were used. Titanium tetraisopropoxide or titanium tetrachloride were hydrolysed in the presence of water and organic (acetonitrile, melamine, monoethanolamine, thioacetamide, thiourea, urea) or inorganic ($(NH_4)_2SO_4$, NH_4SCN, Sb_2S_3, Se_2S_3, SnS_2) compounds. Titanium hydroxide was also used for impregnation with urea in water. The calcination in air was the final step of each preparation.

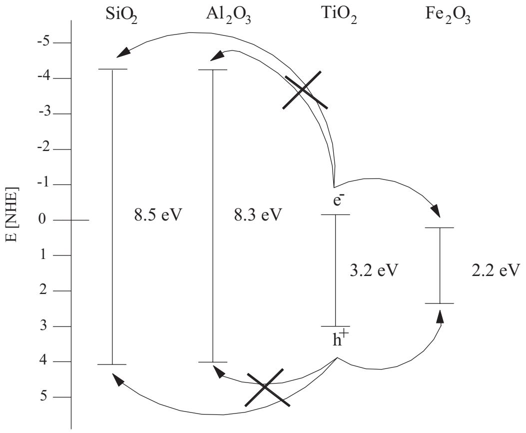

Figure 9. Proposed mechanism of inhibition of electron/hole pairs recombination.

The photocatalytic decomposition of phenol was carried out in the presence of modified and unmodified TiO_2 under visible light (λ>400 nm) for 120 min. Blank experiments showed that phenol was not degraded without a catalyst or illumination. The degradation efficiency of phenol under visible light for catalysts modified with organic dopants is presented in Figure 10.

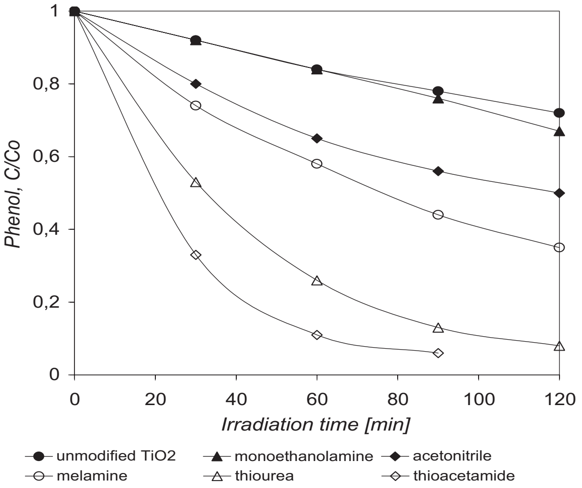

Figure 10. Phenol degradation efficiency as a function of irradiation time for catalysts modified with organic compounds (20 mg/dm^3 of catalyst, λ>400 nm, 1000 W lamp - Io (400-520 nm) = 3.1•10^{-5} Einstein 1/s•cm^2).

The results pertaining to phenol degradation efficiency in the presence of titanium dioxide modified with inorganic compounds are presented in Figure 11.

Among all synthesized catalysts, powder modified with thioacetamide showed the highest activity in phenol degradation for λ≥400 nm. After 60 min irradiation over 80% phenol was degraded. The same time of irradiation in the presence of thiourea-modified TiO_2 resulted in 70% of phenol degradation.

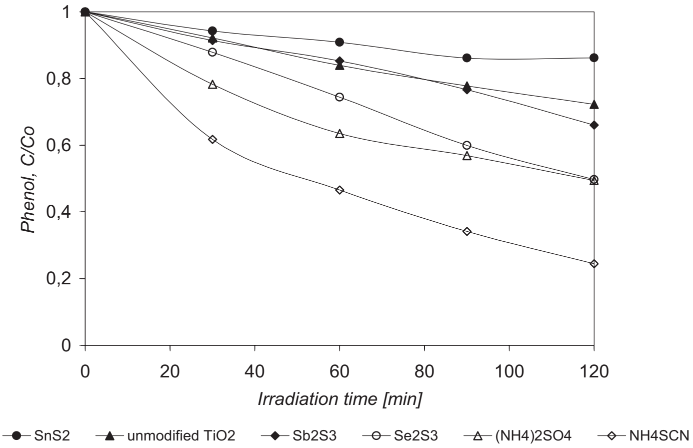

Figure 11. Phenol degradation efficiency as a function of irradiation time for catalysts modified with inorganic compounds (20 mg/dm^3 of catalyst, λ≥400 nm, 1000 W lamp - Io (400-520 nm) = 3.1•10^{-5} Einstein 1/s•cm^2).

The model compound 4-chlorophenol was also used. Two catalysts: TH/N-TiO_2 (prepared from $TiCl_4$ and thiourea) and UR/N-TiO_2 (prepared from $Ti(OH)_4$ and urea) were submitted to the photoactivity tests. The results are shown in Figure 12.

Approximately 50% reduction of TOC was observed for surface modified TiO_2 with urea. After 3 hours of irradiation nearly 30% of 4-CP was degraded using bulk modified TiO_2 with thiourea.

The catalyst modified with thioacetamide and titanium tetraisopropoxide in water contained 0.035 wt.% N, 0.21 wt.% C and 0.016 wt.% S. TH/N-TiO_2 contained 0.3 wt.% N, 0.16 wt.% C and no sulfur was detected.

The XRD pattern confirmed a homogenous crystalline phase of anatase for both catalysts. Diffuse reflectance spectra revealed that the modified catalysts absorb visible light.

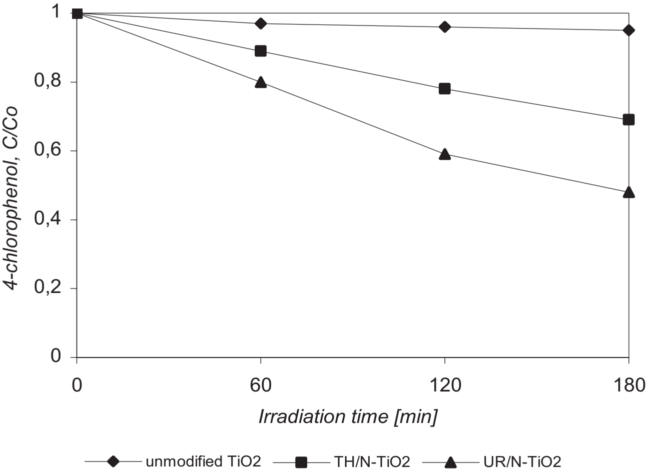

Figure 12. 4-chlorophenol degradation efficiency as a function of irradiation time for catalysts modified with thiourea and urea (1 mg/dm^3 of catalyst, $\lambda \geq 455$ nm, 150 W lamp - Io (400-520 nm) = $2 \cdot 10^{-6}$ Einstein 1/s•cm^2).

3.4 Degradation of Pesticides in an Actual Wastewater

The impact of scale change on the degradation efficiency is illustrated using actual leachate from industrial landfill site. The landfill contains thousands of tons of semiproducts and preparations of chlorinated pesticides which originated from organic synthesis works. Raw and pretreated (after sedimentation and coagulation with $Fe_2(SO_4)_3$) wastewater was photo-degraded in laboratory and in gas-sparged reactors to examine the UV/H_2O_2/air system efficiency and to determine oxidant dose and the reaction time. Pesticide concentrations were determined using high performance liquid chromatography with UV/VIS detector after preconcentration by solid phase extraction.

Typical results of efficiency of pesticides removal are shown in Figure 13.

An 80% efficiency of pesticides degradation was achieved after only 10 min irradiation. During treatment in the UV/H_2O_2 system, the dose of hydrogen peroxide controls the process efficiency. At high concentration (>0.016% v/v), hydrogen peroxide acts as trap for radicals and thus competes with the pollutant resulting in lower degradation rates, see

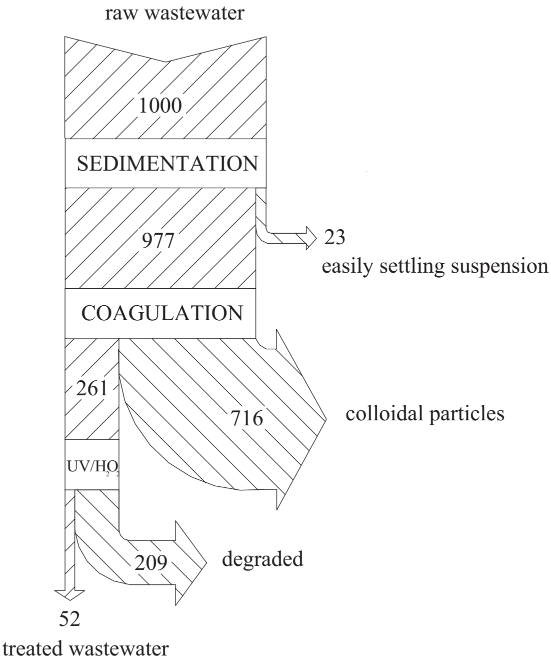

Figure 13. Sankey plot showing efficiency of pesticides removal from industrial dumpsite leachate (photodegradation: UV/H_2O_2 (0.008% v/v) with preliminary sedimentation and coagulation using 40 mg Fe^{+3}/dm^3.

equation 1. At low H_2O_2 concentration (0.08% v/v), absorption of UV radiation becomes insignificant causing impaired generation of hydroxyl radicals.

$$HO^{\cdot} + H_2O_2 = HOO^{\cdot} + H_2O \qquad (1)$$

The results of the scale-up investigation carried out in the ASH reactor are presented in Table 2. In case of laboratory experiments, 10 min irradiation resulted in an average 83% degradation efficiency. For the same reaction time near total pesticide destruction was obtained in the ASH reactor. The investigation in the gas-sparged reactor showed comparable elimination efficiency to the laboratory scale for a twice shorter time (5 min irradiation), although the irradiation power per volume was seven times less than in the laboratory reactor (see Table 2). Higher degradation efficiency achieved in the gas-sparged reactor was caused by reactor design allowing irradiation of only a 3-5 mm thick liquid layer (Bokotko and Hupka, 1996). Analysis of air sparged through the aqueous phase showed that less than 0.1% of impurities were removed due to stripping.

Table 2. Photodegradation of pre-treated wastewater – scale comparison.

Compound/ Parameter	Concentration [μg/dm^3]		Concentration [μg/dm^3] after UV/H_2O_2 (0.008%v/v)		
	Raw wastewater	Wastewater after pre-treatment*	Lab-scale Irradiation time 10 min	Irradiation time 10 min	Irradiation time 5 min
Total amount of organochlorine pesticides	144	0.3	<0.1	<0.1	<0.1
Total amount of organophosphate pesticides	364	11	2	0.7	2
Total amount of other pesticides	11.6	0.2	<0.1	<0.1	<0.1
Total	519.6	11.5	2	0.7	2
Photodegradation efficiency [%]			82.6	93.9	82.6

*After coagulation and adsorption

Another set of experiments was made using TiO_2 supported on hollow glass microspheres. A very high concentration (40 mg/dm^3) of three chlorinated pesticides was used. The amounts exceeded their solubilities in water; therefore, the pesticides were present as very fine, non-settling suspension. Similarly to the results obtained in the UV/H_2O_2 system, the amount of radiation energy was much less in the large scale apparatus than in a small reactor, compare data in Table 3.

Table 3. Comparison of gas-sparged reactor system and laboratory setup for pesticide degradation (initial concentration of each pesticide: 40 mg/dm^3, irradiation time: 150 min, irradiation source: medium pressure mercury lamp, catalyst form: TiO_2 supported on microspheres).

Processing conditions		Gas-sparged reactor system	Laboratory setup
Aqueous phase		Tap water	Distilled water
Volume [dm^3]		80	1
Power of radiation source [W]		2000	150
Irradiation power per reactor volume [W/dm^3]		31	150
Gas phase		air	Oxygen
V_G/V_L [dm^3/dm^3]*		150	50
Elimination efficiency [%]	DMDT	99	99
	p,p'-DDT	94	95
	lindane	50	68
Initial pH		8.0	11.0

*V_G- gas phase volume [dm^3], V_L- liquid phase volume [dm^3]

4. FINAL COMMENTS

Photocatalytic degradation using a UV-enhanced TiO_2 catalyst system is known to have many important advantages, in particular: a large number of organic compounds dissolved or dispersed in water can be completely degraded. Several processing strategies aimed at eliminating persistent organic pollutants from water presented in this work indicate that advanced oxidation technologies can provide protection for groundwaters by treatment of industrial effluents.

Anatase in the UV/TiO_2/oxygen system was more photoactive than rutile which confirms data from the literature pertaining to various organic compounds. Advantageous impact of oxygen on the degradation efficiency was shown.

Since powdered anatase or rutile can adhere to the reactor or lamp surface and be difficult to separate from the aqueous phase, easily separable TiO_2-modified microspheres were successfully used.

It was shown that photodegradation of organic pesticides in industrial wastewater in a UV/H_2O_2/air system can compete with TiO_2-based heterogeneous catalysis, particularly in process scale-up.

Unlike in other aqueous streams, pollutants in underground water require rather more complete degradation, than do streams directed to biological treatment systems. But water treatment by photocatalysis will be the final step, preceded by e.g. stripping or coagulation/flocculation. Thus further research is needed with respect to more favorable kinetics and visible light photocatalysis of dilute solutions or suspensions of contaminants.

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NEW HORIZONS IN PURIFICATION OF LIQUIDS

Novel Colloidal and Interfacial Strategies to Remove Hazardous Molecules, Viruses and Other Microorganisms from Water

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Abstract: The effective, affordable removal of hazardous molecules, viruses and bacteria from water is of paramount importance. In this paper, we investigate the use of various novel colloidal and interfacial strategies for such removal. The objective of this work is to develop methodologies for the removal, in a cost-effective manner, of bacteria and viruses from water, with the implication that such methodologies will be applicable to poorer countries which may not be able to afford the more expensive water treatments such as reverse osmosis. We investigate four different treated filter media (microporous polypropylene filters, fabric filters, diatomaceous earth filters and sand filters) for the removal of 60 nm (negatively charged) polystyrene particles, viruses, and bacteria. The filter media are coated to change their surface properties so that they will have a positive surface charge, which will promote Coulombic interactions between the filter media and microbes. Some of the treatments include coating microporous filters with dimethyldioctadecyl ammonium bromide (DDAB), coating of microdenier polyester textile fabric with DDAB or iron hydroxide to impart a positive charge on the surface, coating of diatomaceous earth with metal oxides, metal peroxides, and metal hydroxides, and coating of Ottawa sand with metal hydroxides for removal of Cryptosporidium. The effectiveness of the treatment on the filter media is determined through measurement of the contact angle, zeta potential, and capture efficiency of bacteria and viruses. The mean pore diameter, the number of filters in the media, and column length of the filter media were determined to be some of the factors that significantly influence the capture efficiency.

Key words: water treatment; virus removal; bacteria removal; microporous polypropylene filters; fabric filters; diatomaceous earth; Ottawa sand; dimethyldioctadecyl ammonium bromide; metal oxides; metal peroxides; metal hydroxides; Cryptosporidium

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1. INTRODUCTION

Water is continually moving around, through, and above the earth as water vapor, liquid water, and ice. In fact, water is continually changing its form. The Earth is essentially a “closed system”, like a terrarium. That means that the Earth neither, as a whole, gains nor loses much matter, including water. This means that the same water that existed on Earth millions of year ago is still here. Thanks to the water cycle, the same water is continually being recycled all around the globe. It is entirely possible that the water you drank for lunch was once used by some other person for bathing.

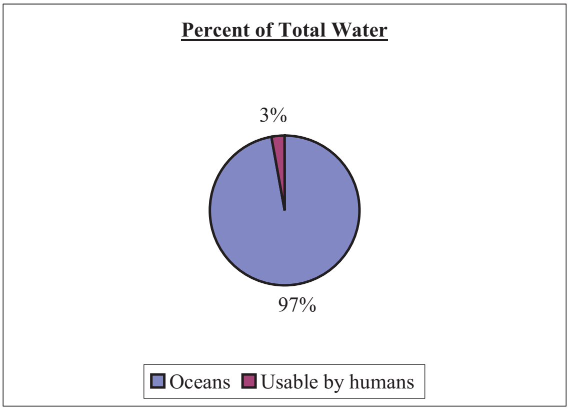


Figure 1. Earth’s Water Supply.

The total volume of water on the earth is an abundant 326,000,000 cubic miles. Approximately 97% of that volume is made up of salt water, which cannot be directly utilized by humanity. Of the 3% remaining, a little more than 2% is locked up as ice in the Polar regions. The remaining water (~0.63%) is found in lakes, rivers, in the atmosphere and underground as fresh water supporting all life forms on earth (See chart below), and much of it is contaminated with various disease-causing microorganisms. Therefore, the development of safe and efficient methodologies for the purification or recycling of water is of paramount importance.

According to a recent report by Johns Hopkins University, nearly half a billion people around the world face water shortages today. By 2025 the number will increase five-fold to 2.8 billion (35 percent of the world’s projected total of 8 billion people). The world’s population, now at nearly 6 billion, is increasing by about 80 million per year. As of 1995, 31 countries, with a combined population of 458 million, faced either water stress or water scarcity (Water Resource Planning, 2001). Although the United States does not currently face critical shortages, there are problem areas:

1. Overall, ground water is being used 25 percent faster than it is being replenished. In particular, the Ogallala aquifer, which underlies parts of six states and irrigates 6 million hectares (14,826,300 acres), has been overexploited and, in some areas, half its available water has been withdrawn.
2. The Colorado River, which flows through several southwestern states, has fed agriculture and enabled rapid growth of desert cities such as Las Vegas, Phoenix, and San Diego. Demands have so drained the river that it no longer consistently reaches its mouth in Mexico's Gulf of California. The river's overuse has been a source of contention between the United States and Mexico.

Before water is available for human use, it undergoes purification treatment. This treatment consists of the removal of contaminants from the water in order to decrease the possibility of detrimental effects on humans and the rest of the ecosystem. The term "contaminant" is used here to refer to an undesirable constituent in the water or wastewater that may directly or indirectly affect human or environmental health. Many contaminants, including a wide variety of organic compounds, metals and micro-organisms (bacteria, virus), are toxic to humans and other organisms.

The use of disinfectants as the final step in water treatment facilities has been quite useful in reducing the number of occurrences of waterborne disease outbreaks. However, it has disadvantages, including the harmful byproducts that may be released to the environment or consumed by humans (Kang, 1998).

1.1 Current Technologies

The most widely used technology for removing turbidity and microbial contaminants from surface water supplies is conventional treatment. This treatment involves the pretreatment steps of chemical coagulation, rapid mixing, flocculation and sedimentation followed by filtration and disinfection (if necessary) (Troyan and Hansen, 1989). Some of the other current technologies that are used for water treatment are listed below:

1. Activated carbon adsorption
2. Ion-exchange resins
3. Diatomaceous earth and sand filters

2. NOVEL COLLOIDAL AND INTERFACIAL APPROACHES

There have been many novel colloidal and interfacial approaches to the removal of contaminants from water supplies. Some of these approaches include coating various filter media (sand, diatomaceous earth, fabric, microporous membranes) with metal hydroxides (Kang, 1998), metal oxides (Kang, 1998; Troyan and Hansen, 1989), metal peroxides (Farrah and Preston, 1985), or cationic surfactants (Kang, 1998). These water treatment procedures are capable of removing bacteria, viruses, and molecules.

Bacteria are single-celled living organisms having an average size of approximately 1000 nm. Viruses are tiny geometric structures that can only reproduce inside a living cell, and range in size from 20 to 250 nanometers. Both bacteria and viruses have a negative surface charge in the pH range of natural waters (Lodder and Liss, 1985). Most filter media also have a negative surface charge in this pH range (Marshall, 1976). Therefore, one would expect it to be difficult to filter bacteria under these conditions due to Coulombic repulsion, unless the surface of filter media is somehow chemically altered to minimize repulsive interactions. It has been found that coatings of metallic hydroxides, oxides, or peroxides on diatomaceous earth or sand significantly increase the zeta potential of these media (Chen et al., 1998). This increase in zeta potential facilitates a greater attraction between the bacteria and filter media (i.e. increases the filter media's adsorption affinity for microorganisms), thereby enhancing filtration efficiency.

Although electrostatic interaction has been reported to be the predominant factor in microbial adhesion to surfaces, external factors such as pH, temperature, and ionic strength have been shown to be important factors as well. Adsorption/adhesion of bacteria generally occurs around the pH range of 3 to 6. At neutral pH, the bacterial cells are negative, so one would expect that lowering the pH acts to decrease the repulsion between the bacteria and the filter surface (Kang, 1998).

2.1 Filtration of Nanoparticles Using Surfactant Coated Filters

The removal of particulate contaminants is very important in many industries, such as water reclamation facilities, water treatment during camping or traveling, microelectronics and pharmaceutical industries. Filtration of particulate contaminants is dependent on several factors including particle size and physicochemical properties of the particles and the filter media. Large particles can be filtered by entrapment mechanism. As the size of the particle decreases, however, particle removal becomes more difficult and thus the interaction between the particles and the filter media must be increased to enhance filtration efficiency.

Deposition of submicron particles onto filters has captured the interest of many investigators over the years (Fitzpatrick and Spielman, 1983; Clint et al., 1973; Spielman and Friedlander, 1974; Rajagopalan and Tien, 1977a, 1977b; Onorato and Tien, 1980; Oak et al., 1985; Shields et al., 1986; Russell et al., 1989; Sisson et al., 1995; Johnson and Lenhoff, 1996; Chang and Vigneswaran, 1990).

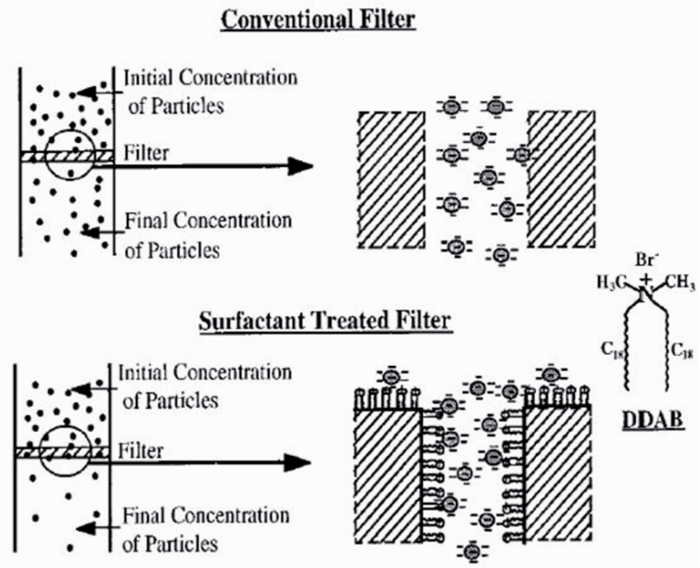


Figure 2. Schematic diagram illustrating particle capturing in conventional filter coated with a monomolecular layer of cationic surfactant.

The main focus of these researches has been on the interactions between the particles and the filters. The dominant factors in submicron particle adhesion/deposition are the surface interaction forces and Brownian motion. (Onorato and Tien, 1980; Russell et al., 1989; Chang and Vigneswaran, 1990) Onorato and Tien (1980) have found that favorable interactions between particles and the filter can significantly increase particle deposition. They reported a 10-fold increase in the deposition of negatively charged particles on a positively charged filter relative to a negatively charged filter. Chang and Vigneswaran (1990) have shown that, by decreasing the electrostatic repulsion between the particle and the filter surface, particle attachment can be greatly enhanced. Harley et al. (1992) studied the adsorption of small particles onto larger particles of opposite charge. Their results support the implication that long-range interactions play an important role in the concentration of particles adsorbed and the rate of adsorption.

We plan to focus on the electrostatic attraction between the particles and the fibers of microporous polypropylene filters. We provide a unique method to lower the energy barrier between the particles and the filter surface and thus increase the deposition of these particles on the surface of the filter. Polypropylene is commonly used to make prefilters and filters because it is extremely inexpensive and very inert. Because most natural materials are negatively charged (Spielman and Friedlander, 1974), we modified the microporous polypropylene filter with a monomolecular layer of cationic surfactant to give it a positive charge. The adsorption of negatively charged particles on the filters and the filtration efficiency of these surface modified filters were studied. This approach is useful not only for removing bacteria, viruses, or nanoparticles in waste water treatment, but can be equally useful in precious metal recovery processes (Figure 2).

2.1.1 Filtration Efficiency of DDAB Treated Filters

Microporous polypropylene filters were coated with a monomolecular layer of dimethyldioctadecyl-ammonium bromide (DDAB) to give them a positively charged surface. DDAB is chosen because of the two hydrocarbon chains that can hydrophobically bond to the surface of the polypropylene and make this surfactant insoluble in water. Contact angle is used as a measure of the amount of DDAB adsorption at the surface of the filters. For contact angle measurements, carbon tetrachloride could not be used because it is too hydrophobic and immediately gets absorbed into the fibers upon contact. Therefore, liquid 1,1,2,2-tetrabromoethane (TBE) was used for contact angle measurements. High contact angle is an indication that the surface is hydrophilic or low contact angle indicates that the surface is hydrophobic (Figure 3).

The average contact angle for the untreated filters is approximately 25° for water/TBE system on polypropylene filter surface. The standard method for the treatment of filters utilized 10 mM DDAB.

The zeta potential of the untreated filters indicates that the filters are slightly negatively charged while the surfactant treated filters exhibited a positive charge for the whole pH range (Figure 4).

Since the untreated filters are slightly negatively charged, it is expected that negatively charged particles will not adsorb on the surface due to the electrostatic repulsion but positively charged particles will adsorb due to the electrostatic attraction.

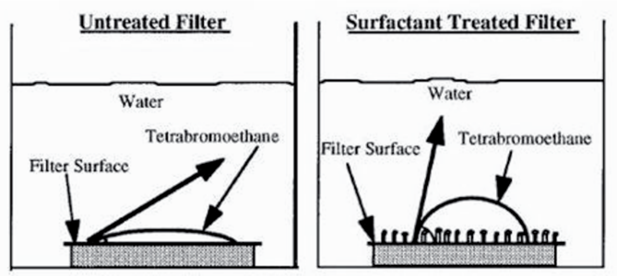


Figure 3. Schematic diagram illustrating contact angle measurement for the microporous filters.

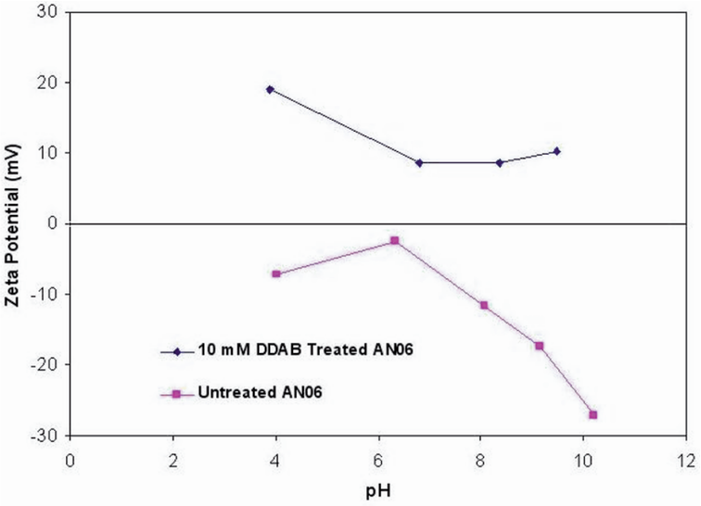
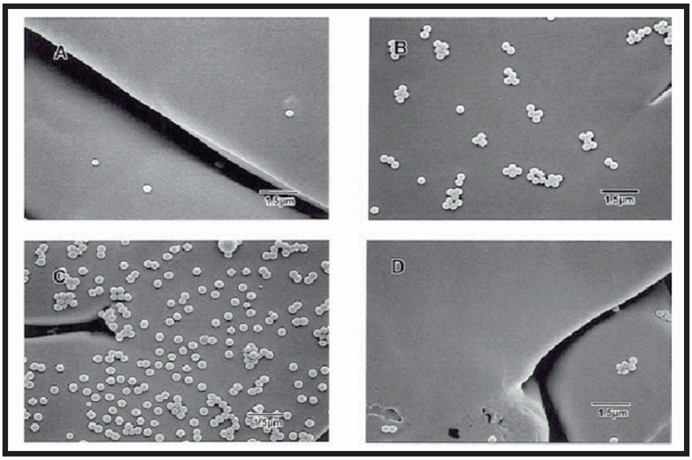


Figure 4. Zeta potential of untreated and 10 mm DDAB treated filters as measured by the streaming potential method.

Figure 5A and 5B show adsorption of negatively and positively charged particles (200 nm) on uncoated filter surface. Figure 5C and 5D show the adsorption of the same particles as 5A and 5B on DDAB coated filter surfaces.



A. Untreated filter and 197 nm negatively charged particles
B. Untreated filter and 200 nm positively charged particles
C. 10 mM DDAB treated filter and negatively charged particles
D. 10 mM DDAB treated filter and 200 nm positively charged particles

Figure 5. (A, B, C, D) Particle adsorption on filter surfaces at pH = 4.0 and 0.012 wt% solids particle concentration.

2.1.2 Effect of Pore Diameter

The capture efficiency of filters with different mean pore diameters was also studied at pH 4.0. Both untreated filters and surfactant treated filters show a sharp decrease in filtration efficiency as the pore diameter of the filters increases (Figure 6).

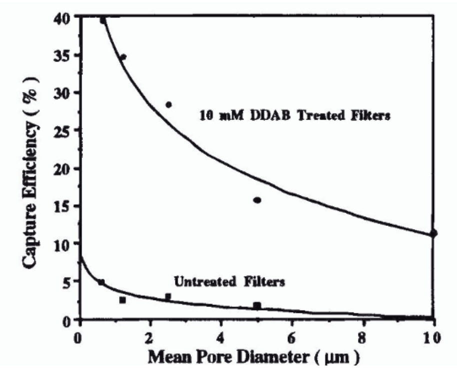


Figure 6. Effect of mean pore diameter on filtration efficiency of filters for 60 nm nanoparticles at pH 4.0 and initial concentration of 0.012 wt% solids.

However, for the 10 mM DDAB treated filters, the capture efficiency remains much higher than for the untreated filters. The decrease in capture efficiency as pore diameter increases is expected, as the electrostatic attraction between the particles and filter surface is only effective within a certain distance. If the particles and surface are too far apart, each will not feel the presence of the other. When the pore sizes are too large, the particles do not have enough time to diffuse to the surface and no interaction takes place. Thus, the particles will pass straight through the filters.

2.1.3 Effects of Multiple Layers of Filters

The polypropylene filters used for these experiments are very thin (150 µm). For this type of filter to be effective in any application, multiple layers of filters must be used. With just four thin filters, more than 95% of the particles can be removed from the solution. As the number of filters increases, the increase in capture efficiency levels off (Figure 7).

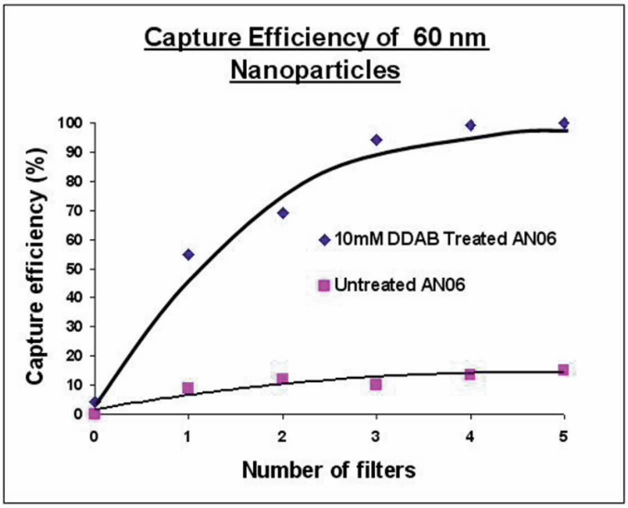
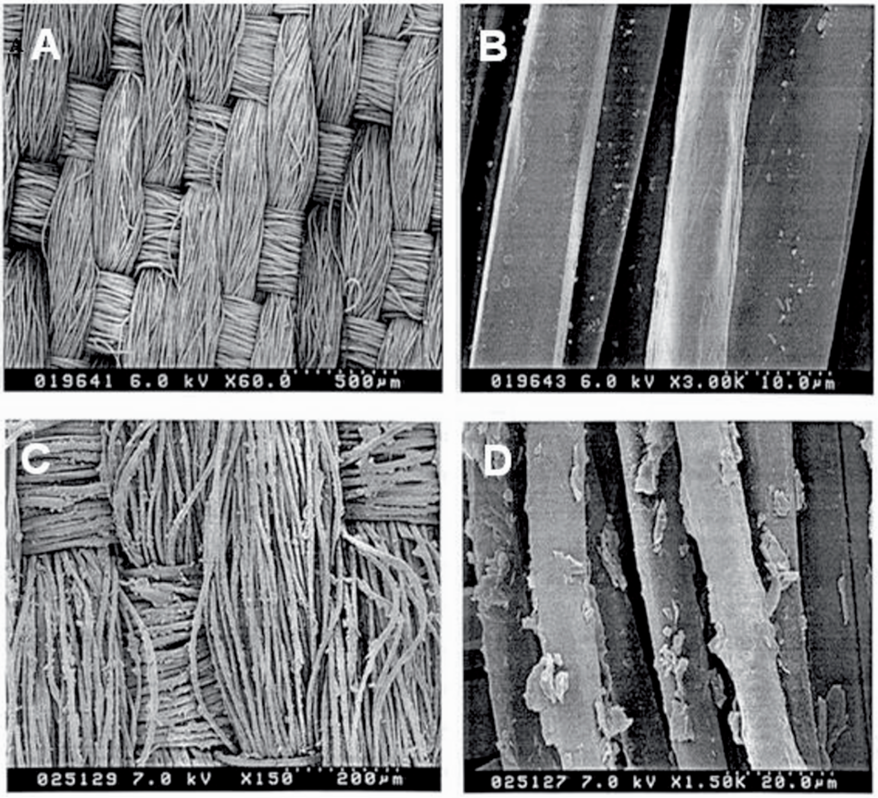


Figure 7. Effect of multiple filters on capture efficiency at pH 4.0 and initial particle concentration of 0.012 wt% solids.

2.2 Coating of Fabrics

The many versatile uses of polyester yarn (e.g. clothing, tire cord thread, conveyer belts, waterproof canvas, fishing nets), its availability, and its tenacity led to the incorporation of microdenier polyester textile fabric as a filtration medium for removal of nanoparticles and biological particles from water (Kang, 1998). The textured and woven surface of polyester increases the tortuosity of the flow path, thereby increasing the probability of particle collision with the filter surface. The fabric was treated with DDAB surfactant to enhance its filtration capabilities and make it more wettable. SEM images of treated and untreated fabric samples are shown below (Figure 8 A, B, C, D) at low Zeta potential measurements revealed that the untreated fabric was negatively charged in the pH range of 4 to 9; while the surfactant treated fabric remained positively charged throughout this pH range. It was also shown that surfactant treatment leads to a decrease in pressure drop across the polyester fabric filters relative to untreated filters



A. Untreated fabric - woven pattern (low magnification)
B. Untreated fabric - individual fibers of woven fabric (high magnification)
C. 10 mM DDAB treated fabric - surfactant coating on woven pattern (low magnification)
D. 10 mM DDAB treated fabric - surfactant coating on individual fibers of woven fabric (high magnification)

Figure 8. Scanning electron micrograph of polyester textile fabric.

during water filtration. This is due to the increased wettability of the fiber surface by adsorption of DDAB. Low pressure drop is desirable because less energy would be required to pass the water through the filters. Kang (1998) also found that increasing the number of filter layers served to greatly increase filtration efficiency for removal of bacteria (*E. coli* and *S. aureus)* (Figure 9).

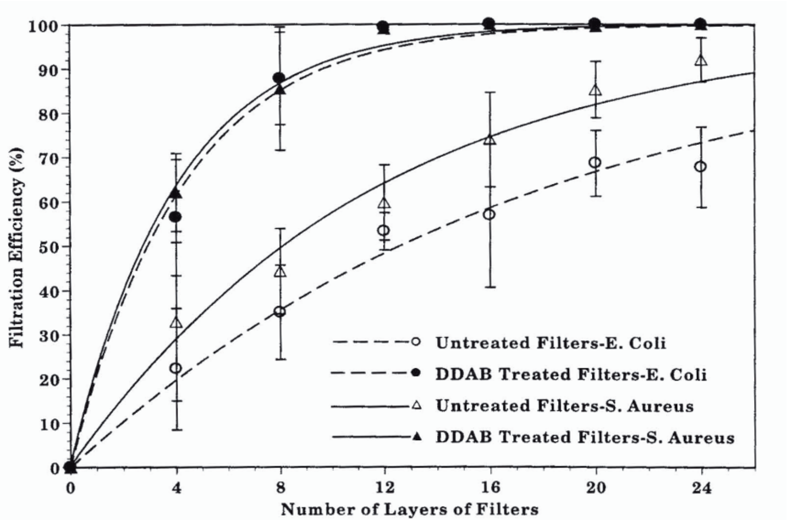


Figure 9. Filtration results of the polyester fabric filters for the removal of E. coli and S. aureus at pH 7.0.

Kang (1998) then extended his study of fabric filters, and instead of DDAB coating, performed experiments with iron oxide coatings. There are sixteen different forms of iron oxides, so in these tests, the form of the iron oxides was controlled to allow for the determination of the effects that each form has on filtration efficiencies. Three forms of the iron oxides (goethite, haematite, and magnetite) were coated on the textile fabric. The zeta potentials of the iron oxide coated filters, although negative, were still less negative than the zeta potentials of the uncoated filters. The coatings substantially increased wettability, which resulted in a reduction in the pressure drop across the filters during filtration. In the filtration of bacteria, *S. aureus* and *E. coli*, the iron oxide coated filters showed some improvement in filtration efficiency (as measured by the filter coefficients). Compared to uncoated filters, the improvements in filtration efficiencies of the goethite, haematite, and magnetite coated filters were 22%, 60% and 53%, respectively for *S. aureus* and 36%, 45% and 51%, respectively for *E. coli*.

2.3 Coated Sand and Diatomaceous Earth

Farrah and Preston (1991) and Farrah et al. (1991) have contributed substantially to the field of knowledge in this area. Their first efforts were focused on the adsorption of viruses by diatomaceous earth coated with metallic oxides (Farrah and Preston, 1991), peroxides (Farrah and Preston, 1991) and hydroxides (Lukasik et al., 1999). Diatomaceous earth was saturated with solutions of aluminum chloride, ferric chloride, calcium chloride, magnesium chloride, or manganese chloride, followed by treatment with deionized water, hydrogen peroxide (for the magnesium oxide sample), or ammonium hydroxide. Studies were performed around pH 7 to 9 to quantify the adsorption of phage MS2, Poliovirus 1 (P1), Coxsackievirus B5 (CB5), and echovirus 5 (E5) onto diatomaceous earth alone and diatomaceous earth in combination with filters. Since metal oxides are slightly soluble in water and are likely to contaminate filtered water with ions or increase the pH of filtered water to objectionable levels (due to formation of metallic hydroxides), the reported results reflect the use of diatomaceous earth coated with manganese oxide or ferric oxide, which are relatively insoluble (Farrah and Preston, 1991). The adsorption results for the diatomaceous earth and filter system with coatings of metallic oxides or peroxides are given in graphical form below (Figure 10) (Note: the adsorptions of CB5 and E5 were negligible, and as such, are not shown):

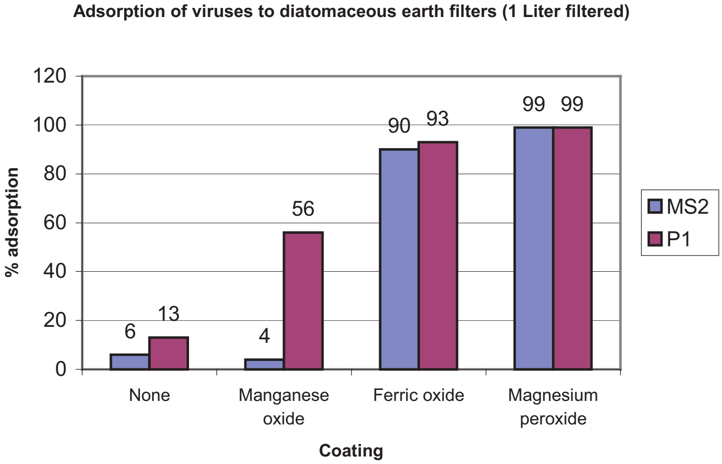


Figure 10. Dechlorinated tapwater with added viruses was passed through two 47-mm filters in tandem that contained 3.5 g of diatomaceous earth (pH 6.9). Values represent the average and range of duplicate determinations.

Farrah et al. (1991) found that using the coated diatomaceous earth in combination with filters serves to increase the filtration capacity relative to coated diatomaceous earth alone. Varying the salts for coating led to an increase in the zeta potential of the diatomaceous earth, which directly reflected the adsorption affinity of the viruses to the filter media (i.e. the more electropositive the zeta potential, the greater the adsorption efficiency will be). It also became evident that combinations of metallic salts (e.g. mixing ferric chloride with aluminum, calcium, and magnesium chloride) provided coatings that significantly increased filtration efficiency relative to coatings with single salts. Eventually, Farrah (Lukasik et al., 1996) decided to expand the studies to consider coating sand with metallic hydroxides. The coating increased the concentration of metals associated with diatomaceous earth and sand particles, their zeta potential, and their ability to adsorb microorganisms in water. The maximum adsorption of microorganisms was achieved using particles coated with a combination of ferric hydroxide and aluminum hydroxide.

As previously mentioned, changing the zeta potential of diatomaceous earth particles greatly increased the ability of the particles to adsorb bacteriophages in batch tests (Figure 11). Increasing the zeta potential to –10 mV permitted a reduction of greater than $2.5\log_{10}$ in the numbers of the three phages tested. Data in Figure 11 were obtained using diatomaceous earth coated with aluminum hydroxides.

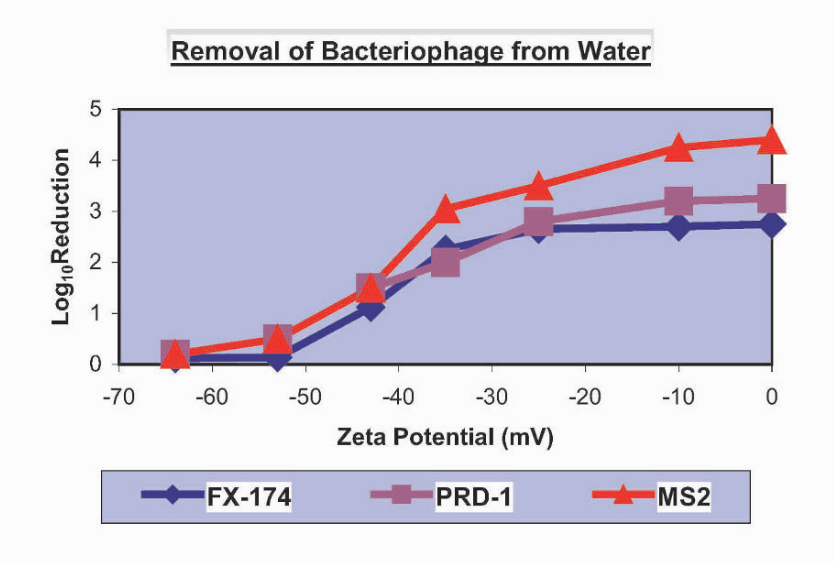


Figure 11. Removal of bacteriophage from water vs. zeta potential of modified diatomaceous earth.

The adsorption of bacteria to diatomaceous earth coated with aluminum hydroxide was similar to adsorption of phages in that increasing the zeta potential increased the adsorption (see Figure 12). It is clear that the zeta potential of the modified solids is directly related to the adsorption of bacteria and bacteriophages. Efficient adsorption of these microorganisms occurs once the zeta potential is raised above approximately –30 mV (for phages) and –10 mV (for bacteria).

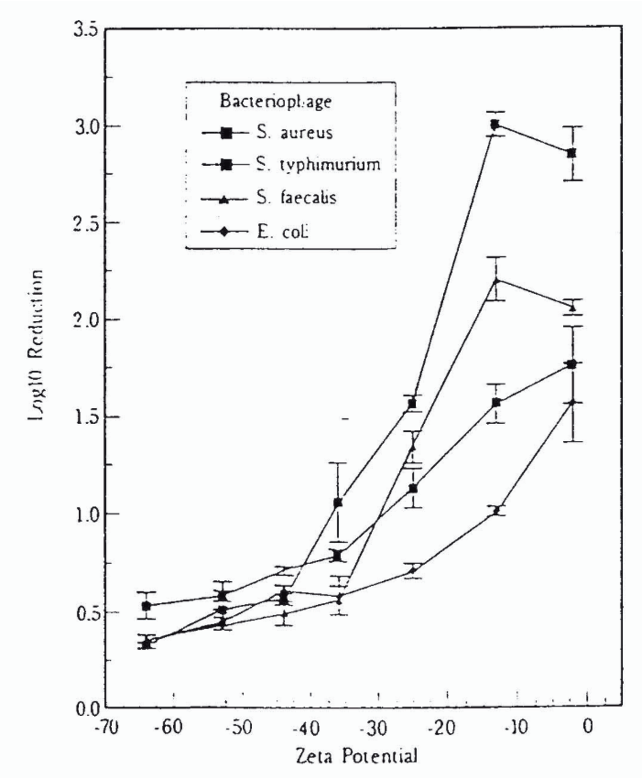


Figure 12. Removal of bacteria (*S. aureus, S. typhimurium, S. faecalis, E. coli*) from water vs. zeta potential of modified diatomaceous earth.

2.4 Removal of *Cryptosporidium* from Water

In another study, (Shaw et al., 2000) the surface coating of hydrous iron aluminum oxide on Ottawa sand was investigated to improve the removal of Cryptosporidium oocysts from water by filtration. Cryptosporidium in water supplies is often a problem in United States and elsewhere. This protozoan causes severe gastroenteritis that is potentially fatal to infants and immunocompromised individuals and that does not respond well to available therapies. Cryptosporidium carries a negative surface electrical charge in the pH range of natural water sources, as do natural filter media such as sand and diatomaceous earth. Thus, removal of the oocysts particles by filtration (without chemical conditioning) is difficult because of electrostatic repulsion between the oocysts and the filter media surface. Coatings of metallic hydroxides, oxides and peroxides on filter media improved the removal of bacteria and viruses from, and reduced turbidity in, water and waste water (Farrah et al., 1991b).

Coating Ottawa sand with the surface coating of hydrous iron aluminum oxide, increased the zeta potential from –40 to +45 mV at pH 7.0, increasing the potential for interaction with negatively charged (-25 mV at pH 7.0) Cryptosporidium oocysts. Water seeded with Cryptosporidium oocysts was passed through parallel columns of uncoated and coated sand at superficial velocities of 200-800 m/d (3.15-14 gpm/sq. ft) and column lengths of 100-400 mm (4-16 in.). In every trial, coated sand removes significantly more oocysts than uncoated sand.

The zeta potential of Cryptosporidium was negative and ranged from -17 mV at pH5.0 to –29 mV at pH8. The zeta potential of the uncoated and coated sand was determined by using streaming potential apparatus. The zeta potential of uncoated sand ranged from 0 mV at pH 2.6 to –55 mV at pH 10.7 with the isoelectric point at pH 2.5 (see Figure 13).

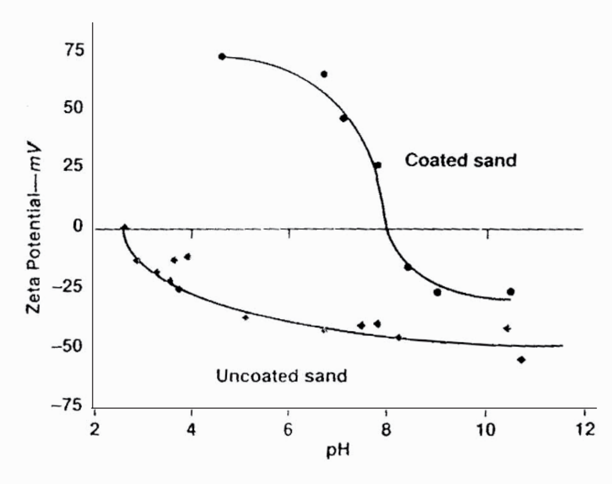


Figure 13. Zeta potential of uncoated sand and sand coated with iron and aluminium (hydr)oxide.

Coating the sand with Fe and Al (hydro)oxides made the zeta potential more electropositive. The isoelectric point of the coated sand was 8.0, which is between the range of 4.6-6.8 for Fe oxides (Fe_2O_3, hematite; FeOOH, goethite) and of 7.8-9.1 for Al oxides (Al_2O_3, corundum; AlOOH, boehmite). The uncoated sand had an Fe content of 0.11 ± 0.026 mg/g sand and Al content of 0.014 ± 0.003 mg/g sand (Table 1).

Table 1. Fe and Al contents of coated and uncoated sand used in various studies.

Sand Size mm(in.)	Coated	Fe (mg/g sand)	Al (mg/g sand)
0.6-0.7(0.02-0.03)	No	0.11	0.01
0.6-0.7(0.02-0.03)	Yes	1.36	1.22

The Al to Fe molar ratio in the coating was 2:1 as compared with 1:1 in the coating solution. The increase in the Al content may be because the Al coating binds better to sand.

The change in the concentration of particles with respect to column length in a granular media filter can be modeled by the first order relation:

$$\frac{\partial c}{\partial z} = -\lambda c \tag{1}$$

in which c is the concentration at length z, and λ is the coefficient that characterizes the filter media. Under conditions in which the coverage of filter media by particles is low enough that it does not affect the rate of particle attachment, this expression may be integrated over the length of the filter column, yielding:

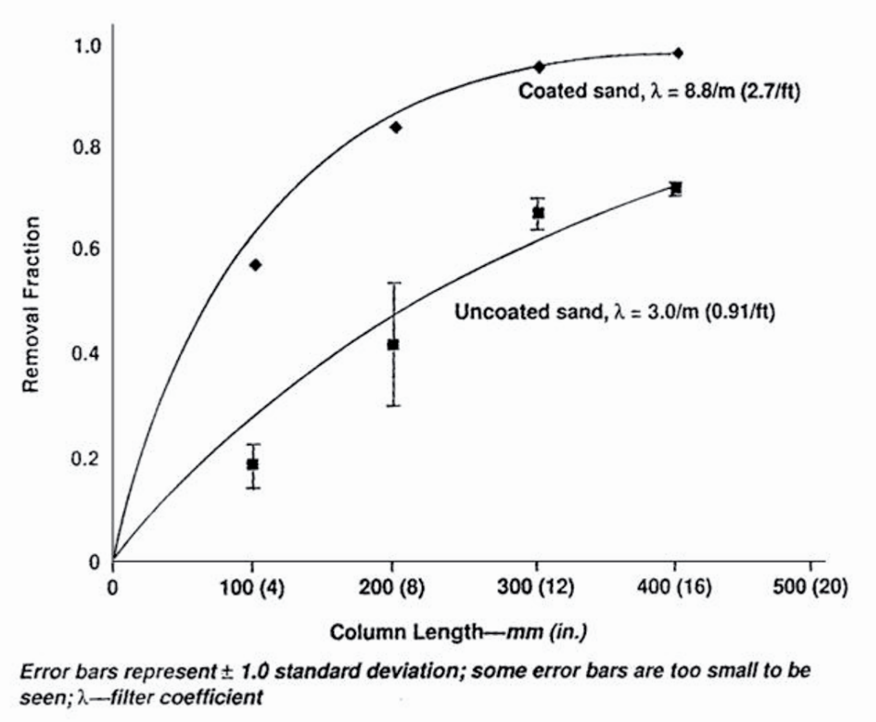
$$\frac{c_{eff}}{c_{in}} = e^{-\lambda L} \tag{2}$$

in which c_{in} is the influent concentration of particles, and c_{eff} is the effluent concentration of particles. The fractional removal efficiency (η) can be expressed as:

$$\eta = \left[1 - \left(\frac{c_{eff}}{c_{in}}\right)\right] = 1 - e^{-\lambda L} \tag{3}$$

Equation (3) indicates that as the filter coefficient increases, the removal efficiency for a given column length also increases. The filter coefficient was calculated by least square nonlinear fit of equation (3) to the curve in

Figure 14. Filter coefficients of coated sand were 2.9 times as high as those of uncoated sand, indicating that coating can significantly improve the removal of Cryptosporidium by granular media filters.



Error bars represent ± 1.0 standard deviation; some error bars are too small to be seen; λ—filter coefficient

Figure 14. Effect of column length on removal of Cryptosporidium.

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FLY ASH-ORGANIC BYPRODUCT MIXTURE AS SOIL AMENDMENT

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Abstract: Greenhouse studies were conducted to assess the potential of fly ash-organic waste co-disposal to enhance crop production. Two pot experiments, one using sorghum sudangrass (*Sorghum vulgaris*) and other using collard greens (*Brassica olreraceae*) were conducted under greenhouse conditions. In addition, a leaching experiment was also conducted to assess the leaching potential of selected heavy metals from fly ash (FA) - sewage sludge (SS) amended soils. The results indicated that biomass production of sorghum sudangrass was significantly increased up to 250 Mg/ha FA: SS mixture amendment, regardless of various ratios of FA: SS mixed amendment. Similarly, the collard greens biomass significantly increased up to 50 Mg/ha. The elemental uptake showed a similar pattern for both sorghum sudangrass and collard greens. The leaching study indicated that increased application rates of amendments (either alone or as mixtures) resulted in increa(sed leaching of metals. Therefore, low to moderate rates of FA: SS mixture (1:1 ratio) could be successfully used as soil amendments.

Key words: sorghum sudangrass; collard greens; fly ash; sewage sludge; soil amendments

1. INTRODUCTION

In the United States, coal fired power plants supply annually more than half of the nation’s electricity demand (ACAA, 1997). Approximately 90%

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of the 890 Mt (million metric tons) of coal produced in the United States in the late 1990s were used by the coal fired power plants (Stewart, 2000). To achieve the national goal of energy independence, the amount of coal consumption is expected to increase rapidly. This could result in large quantities of coal combustion byproducts (CCBs) that will need to be disposed and/or utilized for alternate purposes. Since CCBs contain a variety of potentially toxic organic and inorganic contaminants, improper disposal and management could cause considerable environmental impacts (Davis and Boegly, 1981). The total amount of CCBs generated annually by the coal-fired power plants in the United States estimated to be 100 Mt; 65% of this amount is fly ash (ACAA., 1997).

Of the total CCBs generated, approximately 1.68 Mt were applied on land affected by mining operations (ACAA, 1998). Coal combustion byproducts have also been used in mine reclamation for acid mine drainage (AMD) prevention and treatment, subsidence control and surface restoration (Butalia and Wolfe, 2000). Coal residue, especially fly ash (FA) application to agricultural land does not supply crop requirements of essential plant nutrients such as N and P. However, it could supply sufficient K, Ca, S, B, Mo and a possible number of other essential micronutrients such as Zn. Alkaline fly ash would also be effective in neutralizing soil acidity. Fly ash though contains variable amounts of certain toxic trace elements (e.g. Cd, Cr, Pb, Ni, B, Mo, and Se), which may limit its potential use for land application (Adriano et al., 1980). Essential micronutrients, if occur in excess, may be no less toxic to plants and soil organisms.

Sewage sludge (SS), also known as biosolids, is one of the major solid organic wastes produced by wastewater treatment plants in cities around the globe. Municipal sewage treatment plants in North America process about 7 Mt, d.m. of sewage per day (OECD, 2004). The application of sludge on agricultural land is a common practice around the world, including the USA. Land application of wastewater or reclaimed water, sludge, and municipal solid waste has been shown to benefit crop production and improve soil quality (McCalla and Peterson, 1977; Page and Chang, 1994; NRC, 1996; Basta 1995, 2000). However to realize these benefits, application of sewage sludge must be conducted in an environmentally sustainable manner (CAST, 1976; Chaney et al., 1977).

Unlike fly ash, sewage sludge characteristically contains high levels of the major plant nutrients, N and P, and is enriched in organic matter. Benefits from sludge application on croplands, however, have to be weighed against the potential hazards associated with certain sludge-borne constituents (e.g. heavy metals, organic contaminants). Retention of sludge-borne heavy metals in soils and their accumulation in plant tissues have caused concerns about their extensive use on cropland (CAST, 1976; Chaney et al., 1977). Furthermore, sewage sludge tends to increase acidity of the soils as a result of protons released from organic matter decomposition and mineralization of NH_4^+-N. Increased soil acidity could cause greater solubility of metals and consequently their enhanced plant availability and leaching potential, particularly in soils with poor buffering capacity (Hooda and Alloway, 1993).

With the implementation of the Water Quality Act of 1972 (Public Law 92-500), the generation of sewage sludge has increased tremendously over the past three decades that resulted in the parallel dramatic increase of its land application: from 20 to 54% of the total sewage sludge generated in the USA from 1972 to 1995 (WEF, 1997). The traditional methods of organic waste disposal (landfilling, incineration, ocean dumping) are being restricted or outlawed due to air and water quality concerns. Thus disposal of ever increasing amounts of sewage sludge and fly ash in an environmentally sustainable manner has become a major challenge.

Historically, the use of fly ash (FA) in agriculture has been based on its neutralizing potential and supply of essential elements such as Ca, B, S, and Mo (Martens, 1971; Page et al., 1979). However, the use of fly ash as an agricultural amendment can be enhanced by blending it with potentially acid-forming organic by-products such as sewage sludge. The latter contains significant amounts of N and P (Adriano et al., 1980). Consequently, fly ash may serve as a composting ingredient, along with sewage sludge. Its basic property should permit the neutralization of the acidic sewage sludge, thereby minimizing the bioavailability of heavy metals and the attendant injury to plants. We hypothesized that combined use of fly ash and sewage sludge for land application could prove a beneficial means of their disposal. Because of the contrasting chemical properties and nutrient contents of fly ash and sewage sludge, land application of both products as mixture can improve soil quality and crop production. This could help to alleviate waste disposal and management problems associated with land application of sewage sludge or fly ash.

This chapter summarizes greenhouse studies conducted to evaluate the effect of fly ash and organic waste mixtures on growth and elemental uptake by sorghum sudan grass (*Sorghum vulgaris*), and collard greens (*Brassica olerecea*). In addition, the results of a leaching study are also discussed.

2. MATERIALS AND METHODS

2.1 Greenhouse Study I

The first greenhouse study was conducted using the surface horizons (0-15 cm) of the Orangeburg loamy sand (fine loamy, siliceous, thermic Typic Paleudult) soil from Aiken, South Carolina. The alkaline fly ash (FA1) was collected from the Coal Fired Power Plant (Beech Island, South Carolina), and the sewage sludge (SS1) was collected from the Berkeley County Water and Sewer Treatment Plant (Goose Creek, South Carolina). The treatment consisted of four FA+SS mixtures (1:4, 2:4, 3:4, 4:4 ratios of FA: SS) and six application rates (0, 124, 247, 371, 494, and 988 Mg/ha) of FA+SS mixtures. These rates were applied to the soil (< 2 mm fraction) on the dry weight basis to make a final weight of 6 kg, thoroughly blended, placed in polyethylene pots and each treatment was replicated 4 times. Each pot was incubated for 8 weeks at the field capacity moisture content and *Sorghum vulgaris* seeds were planted (10 per pot). After two weeks, the seedlings were thinned to five plants per pot and grown for 10 weeks. The above ground portion of each plant was harvested 10 weeks after planting, and processed for biomass production and chemical analysis. At the time of harvesting soil samples were also collected and processed for chemical analysis.

Table 1. Selected properties of FA and SS used in this study.

Characteristics, elements (mg/kg)	Materials used				
	FA1	FA2	SS1	SS2	SS3
pH	8.1	11.4	5.7	6.6	6.2
P	200.	232	3600	21300	18500
K	506.	56.8	6020	1760	1460
Ca	4100	8435	2310	15036	18900
Mg	218	4660	872	1759	2570
Mn	13.0	138	110	326	303.
Fe	199	13510	619	21100	17200
B	25.0	299	0.5	27.1	18.1
Cu	19.0	<0.5	71.0	245	247
Zn	6.0	25.6	81.0	510	597
Pb	1.3	2.5	29.0	4.9	2.5
Cd	0.3	2.8	3.5	7.2	10.4
Ni	2.3	14.4	25.0	10.3	19.6
Cr	3.7	26.8	23.0	58.9	27.4
Location	Beach Island SC	Savannah GA	Charleston SC	Savannah GA	Savannah GA

All elemental concentrations are measured on acid digest by ICP-OES and expressed in mg/kg (on dry weight basis).

Table 2. Selected properties of soils used in this study.

Characteristics	Soils		
	Candler	Orangeburg	Ogeechee
Sand, g/kg	967	920	860
Silt, g/kg	8	30	100
Clay, g/kg	25	50	40
pH	7.0	5.4	5.1
Organic matter, g/kg	13.0	11.0	18.5
CEC, cM_c /kg	2.2	2.4	3.9
Texture	Fine sand	Loamy sand	Loamy sand
Vegetation	Citrus	Natural [a]	Natural
Location	Polk, FL	Aiken, SC	Savannah, GA

[a] Natural vegetation includes oak, pine and sweet gum.

2.2 Greenhouse Study II

The second greenhouse study was conducted using surface horizons (0-15 cm) of Candler fine sand (sandy, hyperthermic, uncoated, Typic Quartzipsamments) from Lake Alfred, Florida [FS] and Ogeechee loamy sand (silicious, thermic Typic Ochragult) from Savannah, Georgia [GS]. The alkaline fly ash (FA2) was collected from the Coal Fired Power Plant (Port Wentworth, Georgia), and the sewage sludge (SS2 and SS3) was collected from Savannah Sewage Treatment Plants (Savannah, Georgia). This study was designed to study the effect of application of varying rates (0, 25, 49, 99 and 148 Mg/ha) of 1:1 mixture of sewage sludge and fly ash on plant uptake and distribution of selected heavy metals in various parts of collard greens *(Brassica oleracea var. acephala)*. Five seeds/pot of collard greens were planted in each pot and grown for 42 days in FA: SS amended soils. At the end of 42-d plant growth, plant parts (aboveground vegetative part and belowground roots) were harvested separately, washed, dried and then ground to pass through 40 mm sieve and digested for the analysis of various elements. The digested plant samples were analyzed for Cd, Cr, and Pb using Perkin-Elmer Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

2.3 Leaching Study

A leaching column study was also conducted to evaluate the leaching potential of various trace elements from FA and SS amended soils. Plaxiglass columns, 32 cm long and 7 cm inner diameter, were used to study the transport of metals from soils with various amendments. A Whatman No.

42 filter paper was placed at the bottom of the leaching column and the soils were packed to a height of 30 cm to attain a bulk density of 1.5 g/cm^3. Three replicate columns were used for each treatment. Appropriate quantities of the amendments were mixed with the top 2.5 cm soil and repacked to attain the same bulk density as above. The amended soil columns were saturated with distilled water and excess water was allowed to drain overnight. A Whatman No 42 filter paper was placed on top of each soil column and, deionized water was applied at a constant rate of 1.5 mL/min, using a peristaltic pump, to facilitate leaching. Leachate was collected in half pore volume (220 mL fractions and analyzed for Fe, Mn, Cu, Se, Zn, Cr and Pb. The quantity of metals leached was calculated using the concentrations of each metal and the volume of the leachate fraction. The cumulative amounts of metals leached in all the leachate fractions were calculated. When the ionic strength of all the treatments approached that of the non-treated control, the leaching study was terminated. The soil from each column was divided into three sections at 10 cm increments, i.e. 0-10, 10-20 and 20-30 cm depth sections, and analyzed for pH and Mehlich-3 extractable metals (Fe, Mn, Cu, Se, Cr, and Pb).

3. RESULTS AND DISCUSSION

3.1 Greenhouse Study I

3.1.1 Effect of Fly ash + Sewage Sludge Mixtures on Plant Biomass Production

There is no clear uniform guidance with regards to the application rate of FA, SS or manure as soil amendments. However, some states follow specific recommendations on the rate of amendments based on the allowable metal loading. The application rates, however, are generally expected to be within 10-50 Mg/ha per application. The FA + SS mixture application rates many times greater than the typical rates were also included, which might be suitable for highly disturbed soils as a single application. The application of 124 or 247 Mg/ha of a FA+SS mixture increased plant biomass by approximately 10-fold for all FA+SS mixture ratios compared to the control soil, which received no nutrients or manure application (Table 3).

Table 3. Biomass production of sorghum sudangrass as influenced by FA+SS mixture.

	FA1: SS1 ratio			
	4:1	2:4	3:4	4:4
Application rate (Mg/ha)	(Biomass (g per pot)			
0	2.93 Ac	3.08 Ad	2.99 Ad	3.11 Ad
124	34.58 Aa	37.74 Aa	37.26 Aa	36.65 Aa
247	29.38 Aa	36.60 Aa	35.60 Aa	36.04 Aa
371	17.06 Ab	26.72 Ab	25.18 Ab	27.87 Aab
494	14.43 Ab	16.99 Ac	19.30 Abc	21.58 Abc
988	7.89 Bc	10.46 Ac	17.92 Ac	13.80 Abc

Means followed by similar letters, upper case across FA: SS ratio comparison and lower case across application rate comparison are not significantly different ($P < 0.05$) according to Duncan's Multiple Range Test (Sajwan et al., 1995).

Table 4. Concentrations of various metals in sorghum sudangrass grown on fly ash: sewage sludge (FA1: SS1) mixture amended soils.

Application		Plant tissue concentration [†]				
Ratio FA: SS	Rate	Mn	Fe	B	Cu	Zn
	Mg/ha	µg/g				
1:4	0	303 c	51 c	8 c	3 b	27 c
	124	513 a	83 a	11 bc	14 a	113 b
	247	421 b	62 b	14 bc	15 a	174 a
	371	318 c	56 bc	15 bc	14 a	176 a
	494	267 c	60 b	19 ab	15 a	154 a
	988	133 d	64 b	25 a	17 a	99 b
2:4	0	298 bc	96 a	10 d	3 c	27 c
	124	375 a	105 a	20 c	13 b	99 b
	247	352 ab	112 a	25 bc	14 b	103 b
	371	300 bc	94 a	27 bc	14 b	136 a
	494	253 c	110 a	33 b	16 a	131 a
	988	159 d	112 a	45 a	13 b	98 b
3:4	0	355 a	61 c	1 e	6 b	24 e
	124	305 ab	99 a	13 d	12 a	95 d
	247	320 ab	91 b	17 cd	12 a	108 c
	371	311 ab	84 b	25 bc	14 a	144 a
	494	287 b	85 b	30 b	14 a	149 a
	988	201 c	96 a	49 a	14 a	122 b
4:4	0	378 a	46 c	1 c	3 c	29 e
	124	259 b	87 b	6 b	12 b	80 d
	247	279 b	82 b	8 b	12 b	102 bcd
	371	270 b	88 b	21 a	14 a	117 ab
	494	251 bc	117 a	22 a	14 a	114 abc
	988	192 c	116 a	25 a	15 a	136 a

[†] Means followed by similar letters, within each metals by each FA:SS ratio, indicate no significant difference according to Duncan's Multiple Range Test ($p<0.05$) (Sajwan et al. 1995).

Excessive mineral salts and B content in the FA amended soil were found to be the major contributory factors for the observed plant mortality and biomass reduction (Mass, 1990; Menon et al., 1993). It would therefore appear that the use of SS or FA alone, at excessive rates, has the potential to restrict the plant growth, possibly by modifying soil pH and soil-nutrient supply. However, the use of FA+SS mixture could provide beneficial effects on crop production (Table 3), particularly when applied at a suitable application rate. The findings also showed that it is beneficial to use FA+SS mixtures compared to FA or SS, and the mixture should not be applied at rates greater than 247 Mg/ha even when designed as a single application.

3.1.2 Effect of FA+SS Mixtures on Plant Tissue and Soil Metal Content

The contents of B, Fe, Mn, Cu, and Zn in the plant tissue at the time of harvest are presented in Table 4. These results revealed that B was the only element that consistently increased with increasing rates of FA: SS additions across all FA: SS ratios. The plant uptake of other elements had a variable response to the FA+SS mixture ratios and application rates (Table 4). One of the possible reasons for the decreased biomass yields when the mixture applied above 247 Mg/ha (Table 4) may have been the accumulation of phytotoxic level of B and mineral salts. The regression analysis (not presented here) carried out between biomass vs. B in the plants and biomass production vs. salt content measured by electrical conductivity (EC) and total dissolved salt (TDS) in the soil confirmed the negative relationship between these factors. This is consistent with other reports of similar observations with regard to the possible phytotoxic effect of B and soluble salts from FA and biosolids (Menon et al., 1993; Adriano et al., 2002). The concentrations of Mn, Fe, Cu and Zn in the plant tissue showed some variations, with no particular trend (Table 4). The concentrations, however, were within their normal ranges. Other trace elements such as Cd, Se, As and Pb in the plants and soil extracts were below the detection limits of the ICP-OES, and therefore are not reported here. The contents of Mehlich-3 extractable Mn, Fe, and B increased with increasing FA+SS application rates and FA:SS mixture ratio, while the concentrations of Cu and Zn were largely unaffected (Sajwan et al., 1995).

3.2 Greenhouse Study II

3.2.1 Effect of soil and SS: FA Mixture Amendments on Plant Biomass Production

The biomass production and the distribution of Cd, Cr, and Pb in various plant parts are presented in Table 5. Soils amended with a mixture of domestic sewage sludge and fly ash had greater biomass production compared to that of the mixture of industrial sewage sludge and fly ash. Irrespective of source amendments, soil type, and plant parts biomass production increased up to 49 Mg/ha application rate and thereafter decreased with further increase of rate of application.

3.2.2 Effect of Soil and SS: FA Mixture Amendments on Metal Accumulation

The accumulation of selected heavy metals (Cd, Cr, Pb) and their distribution increased with increasing rates of amendment at least up to 49 Mg/ha and thereafter the differences were not that much with further increase. It was also interesting to note that the greater accumulation of Cd, and Cr was observed in aboveground portion of collard greens compared to belowground portion regardless of the source of the sewage sludge. The Cd was below the detection limit in belowground plant parts. Result of this study indicated that one should be very careful in using these amendments for growing leafy vegetables such as collard greens.

Table 5. Dry matter and selected heavy metal concentrations in collard greens plant parts.

Soil	Rate of Amend-ment Mg/ha	Sewage sludge (SS2) from domestic source and fly ash (1:1) ratio							
		Aboveground part				Belowground part (roots)			
		g	mg/kg			g	Mg/ kg		
		Dry matter	Cd	Cr	Pb	Dry matter	Cd	Cr	Pb
FS	0	1.70	0.87	0.65	0.62	0.20	0.00	0.63	0.38
	25	3.03	1.83	3.67	4.47	0.30	0.00	0.83	1.63
	49	3.30	2.23	4.73	5.10	0.43	0.00	0.87	3.27
	99	2.53	2.07	4.67	4.93	0.40	0.00	0.87	3.93
	148	2.47	2.07	4.67	4.97	0.30	0.00	0.83	3.83
GS	0	2.03	1.12	0.72	0.92	0.20	0.00	0.53	0.63
	25	2.63	2.17	3.60	5.47	0.27	0.00	2.03	2.73
	49	3.20	2.40	4.83	5.53	0.46	0.00	2.10	4.00
	99	1.77	2.28	4.80	5.47	0.29	0.00	2.70	3.98
	148	1.43	2.23	4.73	5.50	0.26	0.00	2.63	3.70

		Sewage sludge (SS2) from domestic source and fly ash (1:1) ratio							
		Sewage sludge (SS3) from industrial source and fly ash (1:1) ratio							
FS	0	0.33	1.07	0.65	0.62	0.10	0.00	0.63	0.38
	25	0.93	2.07	3.67	4.53	0.13	0.00	2.83	6.63
	49.	1.20	2.11	4.60	5.51	0.40	0.00	3.07	7.67
	99	1.13	3.09	5.09	5.34	0.34	0.00	2.98	6.87
	148	0.97	2.96	4.80	5.31	0.26	0.00	2.83	6.33
GS	0	0.53	1.12	0.72	0.92	0.13	0.00	0.53	0.63
	25	2.23	2.18	3.68	4.09	0.17	0.00	1.71	2.73
	49	2.60	2.60	4.98	7.60	0.40	0.00	2.87	9.13
	99	2.43	2.80	4.84	6.87	0.37	0.00	2.93	8.20
	148	1.23	2.78	4.80	6.82	0.33	0.00	2.82	8.16

FS (Florida Soil), GS (Georgia Soil), (Sajwan et al., 2005)

3.3 Leaching Study

The results of this study indicated that leaching of most elements except Cu, increased concurrently with the rate of FA+SS mixture application up to 99 Mg/ha (Table 6). A further increase in FA+SS application rate to 148 Mg/ha had little effect on the leaching of most metals. The amount of Cu in the leachate was the lowest compared to the other metals and the rate of amendment had little or no effect on the amount of Cu leached from this soil (Table 6). The quantities of metals leached from the FA+SS mixture application rate of 148 Mg/ha were lower than combined quantities of the metals leached from the soil column, which received 74.1 Mg/ha of either FA or SS. It has been generally accepted that the reaction of the soil system (soil pH along with redox) mainly governs solubility of metals (Lindsay, 1979). Amending soils with varying amounts of FA, SS or FA+SS mixture may modify the pH of the soil, which in turn, could affect the leaching potential. This depends on the composition of the FA and SS materials and the buffering capacity of the soil.

Table 6. Total quantities of metals (μg) in 1540 mL of leachate from the Orangeburg loamy sand amended with FA2 and SS2 mixture and FA2 or SS2.

Treatments Mg ha^{-1}	Cr	Zn	Cd	Cu	Pb	Ni	Fe	Mn
25 (FA+ SS)	203	85	63	43	483	174	38	3000
49 (FA + SS)	219	87	99	38	613	217	65	5125
99 (FA + SS)	241	100	106	45	812	268	345	10192
148 (FA + SS)	166	109	95	41	686	224	930	12561
74 (FA)	163	92	85	39	671	216	57	5166
74 (SS)	200	126	101	60	909	277	1383	11114

Source: Alva et al. (1999a, 1999b).

4. GENERAL DISCUSSION AND CONCLUSIONS

Results of greenhouse studies demonstrated that plant establishment, subsequent growth and biomass production of sorghum sudangrass were not affected significantly up to 124 or 247 Mg/ha FA:SS mixture amendment, regardless of various ratios of FA:SS mixed amendment. However, for collard greens the biomass was not significantly affected up to 25 or 49 Mg/ha. Therefore, it is difficult to generalize this trend without extensive studies on a wide spectrum of plant species. It should be stressed that some of the high rates of FA+SS mixture used in these case studies would not be considered logical even for a single application.

It should be noted that the modification of soil pH could adversely affect soil-nutrient supply as well. Increased application rates of these amendments (either alone or as mixtures) resulted in increased leaching of metals in the column studies. However, the quantities of cumulative leaching of the metals from the soil amended with 1:1 FA+SS mixture, at a high rate (148 Mg/ha) were substantially lower than those combined from soil columns amended with 74 Mg/ha each of FA or SS as single amendments. This clearly showed the benefit of using FA+SS mixture rather than using them separately. The concentration of most metals in the leachate following application of low to moderate rates of FA+SS mixtures generally did not exceed the USEPA stipulated maximum contaminant level for most metals.

Furthermore, the increased accumulation of B in plant tissue, observed in both greenhouse studies following the use of FA+SS mixture was considered as the possible reason for the reduction in biomass production. Overall these studies showed that mixture of FA+SS (1:1) as a soil amendment could provide benefits in terms of soil fertility without any significant risk of soil, water or plants being contaminated, particularly when applied at reasonable rates.

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Biosystems for Non-Destructive Remediation and Immobilization of Pollutants in Soils, Sediments and Detoxification of Industrial Wastes

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PHYTOREMEDIATION AND PHYTOTECHNOLOGIES: A REVIEW FOR THE PRESENT AND THE FUTURE

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Abstract: Research on phytoremediation and phytotechnologies is proceeding at a different pace in the EU than in the USA. In fact although EU researchers have closed the gap on basics and fundamentals of phytoremediation with overseas researchers, a large gap of application exists in the extent of cases to which phytotechnologies are applied. The purpose of this paper is to review weaknesses and strengths of European contribution in the field. Research in the field of phytoremediation can be classified into two main groups: understanding of basic mechanisms and knowledge implementation for applications. Many groups are actively working to elucidate the basic genetic, molecular and cellular mechanisms underlying accumulation, transport and tolerance of plants to heavy metals. In comparison, interest towards organic pollutants is less widespread. Excellence in European research concerns the basic enzymology of phytodegradation of PAHs, TNT, dyes, herbicides, studied by means of in vitro cultures or in whole plants. As far as applications are concerned, the favorite phytotechnology in Europe is definitely the constructed wetland, often planted with *Phragmites australis*, which has been used for treatment of mine wastes, explosives, agricultural and municipal wastewaters. Other favored phytotechnologies are phytostabilization and phytoextraction. Phytotechnologies are particularly useful for preservation of agricultural soil. Cutting downstream the flow of pollutants into the food chain is a strategy also for conserving food safety and human health. Since research on phytotechnologies involves hundreds of scientists and dozens of research institutions and private companies; communication and networking are very important. Training and formation represent also an important aspect in developing a greater comprehension and acceptance of phytoremediation.

Key words: phytoremediation; Europe; research activities; new trends; networking

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1. INTRODUCTION

Phytoremediation, defined as the use of vegetation for in situ treatment of contaminated soils, sediments and water, is an environmental biotechnology that has attracted recently the interest of scientists, public opinion, regulators, and public administration. Several extensive reviews and books have been written on this topic. As a book, a very extensive treaty is "Phytoremediation: Transformation and Control of Contaminants" edited in 2003 by McCutcheon and Schnoor, and as a review the most recent is by Elizabeth Pilon-Smits (2004). The purpose of this paper will be to review the most recent advances in this field, as they have been gleaned by meetings of the EC COST Action 859 "Phytotechnologies to promote sustainable land use management and improve food safety", which took place from late 2004 through spring 2005 in Europe. In these meetings, the richness and diversity of research in European phytoremediation has emerged, a field in which US research has been one step ahead.

1.1 Phytotechnologies and Their Application

The traditional term of phytoremediation has been recently supplanted by the term "phytotechnologies", used to indicate all applications in which plants are used to manage and control pollutants, even without removing or destroying it (ITRC, 2001).

Phytotechnologies are based upon the basic physiological mechanisms taking place in higher plants and associated microorganisms, such as transpiration, photosynthesis, metabolism, and mineral nutrition. Plants dig their roots in soils, sediments and water, and roots can take up organic compounds and inorganic substances; roots can stabilize and bind substances on their external surfaces, and when they interact with microorganisms in the rhizosphere. Uptaken substances may be transported, stored, converted, and accumulated in the different cells and tissues of the plant. Finally, aerial parts of the plant may exchange gases with the atmosphere allowing uptake or release of molecules. A series of six phytotechnologies have been identified (ITRC, 2001) which may address different contaminants in different substrates, and which rely on one or more of the plant properties as in the exemplification listed above:

1. phytotransformation, ideal for organic contaminants in all substrates
2. rhizosphere bioremediation, applied to organic contaminants in soil
3. phytostabilisation, for organic and inorganic contaminants in soil
4. phytoextraction, useful for inorganic contaminants in all substrates
5. phytovolatilisation, which concerns volatile substances
6. evapotranspiration, to control hydraulic flow in the contaminated environment

From knowledge of the basic phytoremediation, applications (phytotechnologies) have been designed which consider the appropriate combination of plant choice and technology to address a specific contaminated site. This list has been taken from the document prepared by ITRC in 2001, even if different authors may identify a different set of methodologies:

1. vegetative covers for control of infiltration
2. vegetative covers for soil remediation
3. hydraulic barriers
4. tree stands for remediation of soil and groundwater
5. treatment wetlands
6. riparian buffers
7. hydroponic systems

These approaches differ in purposes and goals, which can be remediation, decontamination, control of water movement and leaching of the contaminant, containment, stabilization. Once the goal is identified, the technique is applied by choosing the appropriate plants and the relevant phytotechnologies. Examples and case studies describing these applications may be found in the specialised literature in books and in journals.

1.2 Phytotechnologies in Europe

Use of phytotechnologies in Europe is limited, as compared with USA and Canada. In those countries, private companies have been formed with the purpose to apply plant resources to control pollution (van der Lelie et al., 2001). European normative and public opinion are still precautionary in the use of phytoremediation. The following constraints have been evidenced:

- due to limited knowledge and poor dissemination, there are doubts in the public opinion and limited acceptance,
- the current regulations, which do not clearly consider phytoremediation within the current applicable technologies
- unfavorable competition with standard clean-up methods, which can provide a long-standing record of success
- lack of sufficient investments, for encouraging the private initiative
- proprietary rights, which may hinder application of approaches already proved as successful in USA

One of the main constraints, however, rests on the lack of fundamental and applied research. In fact, research on the basic mechanisms of phytotechnologies and case studies are still needed in order to use these technologies in a coherent way. Moreover, in the last Framework Programme of the European Commission there was no topic really suitable for presentation of projects on phytotechnologies, and most of the European scientists have to rely on small budgets obtained from National or Regional sources. Nevertheless, the EC has recently funded projects on this topic, as listed in the CORDIS web site (www.cordis.lu), which have avoided a complete blockage of research in phytoremediation. Another tool which was instrumental to maintain interest in phytoremediation and phytotechnologies has been the COST Action 837 "Plant biotechnology for the removal of organic pollutants and toxic metals from wastewaters and contaminated sites" and the new one, 859, previously mentioned.

A survey, carried out in April 2005, enlisted all scientists involved in phytotechnology research in Europe which could be found in publications indexed by ISI (Current Contents) and/or those who participated to the COST meetings (see COST Actions www sites, lbewww.epfl.ch/COST837/ and www.gre.ac.uk/cost859/). The countries involved were 29, including Israel and Turkey which participate to European projects. About 350 research groups were involved, of which 60% were in Universities, 30% in research institutes and 10% in private companies. Indeed this distribution of efforts may be biased by industrial research which, for competition problems, is limited in dissemination through journals.

From papers published in 2001-2004, the distribution of topics for research is the same in Europe as in the USA, with most of the efforts concentrated on understanding the basic principles of phytotechnologies. A new trend is in the field of management and sustainability of enabling technologies. At this purpose different papers concern risk assessment and utilization of Decision Support Systems.

Here follows a short review of recent results obtained by European scientists, focused on the two main topics of principles of phytotechnologies and implementation and use of phytotechnologies.

2. BASIC PRINCIPLES OF PHYTOTECHNOLOGIES

2.1 Basic Principles – Heavy Metals and Inorganic Contaminants

2.1.1 Uptake and Transport

Interaction among plants and metals starts in the root environment. All phytotechnologies can be applied only if the contaminant is in contact with roots, and most of them rely on contaminant uptake by roots. This is the reason why plasma membrane transporters are a subject of research for phytotechnology implementation. Heavy metals uptake involves the same kind of transporters which otherwise provide macro- and micronutrients entrance. Recently, Perfus-Barboech et al. (2002) have demonstrated the involvement of Ca channels in Cd uptake in *Arabidopsis thaliana*. The possibility of Cd mimicking Ca in plant cells can also justify its toxicity with perturbation of metabolism and homeostasis of this vital element.

Studies performed with plant cell protoplasts have tried to ascertain if differences in transport in sink tissues could explain the different behavior of hyperaccumulator plants (Cosio et al., 2004). The results obtained with *A. halleri* and *Thlaspi caerulescens* show that plasma membranes of leaf cells do not account for differences in transport. Therefore, it has been hypothesized that other mechanisms may be active to direct the metals to their subcellular compartments, where they are stored: vacuoles and lignocellulosic material such as cell wall may be among these.

Studies of metal transport, and especially in the case of radionuclides, can benefit from autoradiographic techniques, as shown by Soudek et al. (2004) with Cs. Imaging techniques allowed comparison between different species for uptake efficiency, but they also revealed potential sink tissues, providing useful information for implementation of phytoextraction.

2.1.2 Accumulation and Sequestration

In recent years, several authors have attempted to describe the differences between hyperaccumulator taxa and non-accumulator congeners by exploiting analytical techniques which give information on speciation and localisation of metals in plant tissues. To understand the molecular bases of the hyperaccumulation capacity and to define the storage strategies will be instrumental in developing and implementing phytoextraction. Analytical techniques based on X-ray emission (scanning electron microscopy and microanalysis) have been used to show Ni accumulation in the leaf trichomes of *Alyssum bertolonii*, in contrast with the non accumulator *Alyssum montanum* which stores Ni in the roots (Marmiroli et al., 2004). *Arabidopsis halleri*, a Zn and Cd hyperaccumulator, has been studied by Sarret et al. (2002). Zn is mainly sequestered in vacuoles of leaf trichomes and mesophyll cells. The authors determined through EXAFS that the two main forms of Zn in the plant roots were malate and phytate (or possibly phosphate), whereas in trichomes it is coordinated by C atoms, presumably belonging to organic acids. Careful computations however suggest that Zn in trichomes, even if highly concentrated, cannot constitute the major sink. Another interesting result concerns Zn binding in the non accumulator *Arabidopsis lyrata*, in which phosphate species were predominantly involved. With a similar approach based on EXAFS it has been possible to show that Pb can be accumulated in roots of walnut trees by coordination with C atoms of cellulose and lignin (Marmiroli et al., 2005).

2.1.3 Identification of Genes and Proteins Involved In Tolerance and Accumulation

To unravel the molecular and biochemical mechanisms of hyperaccumulation, the search for genes and proteins is being carried on with genomics and proteomics approaches. Genomic efforts have been promoted by the EC in research projects studying the properties of the hyperaccumulators, and results are showing how different genes are induced by metals in these plants and their congeners (van de Mortel et al., 2004). Comparative genomics and proteomics also contribute knowledge on orthologous genes, novel sequences, and molecular markers (Tuomainen et al., 2004).

Bernard et al. (2004) have shown how ectopic expression of *Thlaspi* genes in yeast led to the isolation of a new gene function involved in Cd transport and probably also hyperaccumulation, a P-type ATPase.

Genetic mapping is the method of choice in the case of quantitative traits, and some authors are building maps of Quantitative trait Loci (QTLs) for hyperaccumulation and tolerance in model plants and in hyperaccumulators. Due to phylogenetic relationships with known hyperaccumulators in the family Brassicaceae, *Arabidopsis thaliana* is the best model plant available, due to the complete knowledge of the genomic sequence and to the genetic knowledge. The group led by Martin Broadley has recently mapped QTL involved in Cs accumulation of *A. thaliana* (Payne et al., 2004). Several accessions were analysed for Cs accumulation, leading to the description of a 2-fold variation in Cs concentration. Crosses among contrasting phenotypes and analysis in segregating progenies led to mapping of putative QTLs on several chromosomes; the existence of two QTLs on chromosomes I and V was confirmed from the analysis of segregating populations from independent crosses. Mapping of candidate genes in these regions will lead to new hypotheses about the structure and function of these QTLs.

2.1.4 Genetic Bases of Tolerance

Classical genetic studies have been exploited in order to address the issue of genetic bases of tolerance and/or accumulation in addition to genomic and proteomic approaches towards gene identification. For this purpose, model plants of choice have traditionally been *Arabidopsis halleri* and *Thlaspi caerulescens*, two hyperaccumulators that can be crossed with non-hyperaccumulator ecotypes or congeners for studies of traits segregation. Genecological observations have demonstrated that tolerance to one heavy metal is a trait which is independent from accumulation of the same metal, and also that tolerance is controlled by few major genes. Interspecific crosses between *A. halleri* and *A. lyrata* ssp. *petraea* have contributed information about tolerance and hyperaccumulation to Cd (Bert et al., 2003): tolerance and hyperaccumulation segregate as independent characters, whereas Cd tolerance co-segregates with Zn tolerance. Moreover Cd and Zn hyperaccumulation seem to be co-regulated or controlled by the same genes.

The same approach has been pursued in *Thlaspi* with ecotypes differing in accumulation capacity (Zha et al., 2004). Segregation results suggest that 2 genes at least are responsible for Zn accumulation, whereas for Cd accumulation more than one gene could be involved. Correlation between accumulation of Zn, Cd and Mn is consistent with a multiple transporter with simultaneous specificity for the three metals. Also in *Thlaspi*, Cd tolerance and accumulation segregate as independent characters.

2.1.5 New Contaminants

Phytotechnologies have traditionally been applied to heavy metals, nutrients and radionuclides. New contaminants of interest include arsenic: only recently have hyperaccumulators for this element been described: the fern *Pteris vittata* (Zhao et al., 2003) and other species of the same genus (Zhao et al., 2002). Phytochelatins play a role in As tolerance, as reviewed in Zhao et al. (2003), but in *Pteris* As is mainly in an inorganic form as arsenite in the vacuole of leaf cells. The authors suggest that phytochelatins may have a role in binding the small As quantities found in the cytoplasm.

Mercury is also attracting new interest, and there are indications that plants may be able to volatilize it as metallic mercury (Ernst et al., 2005).

2.2 Basic Mechanisms – Organic Contaminants

2.2.1 Mechanisms of Genetic Controls – Candidate Genes

As in the case of inorganic contaminants, also for organic contaminants researches are focusing on gene identification with a genomic approach. Recent advances concern specific candidate genes, coding for enzymes which are known to be involved in the metabolism of xenobiotics. The best known example is glutathione transferase (GST), a multigenic family recently reviewed by Frova (2003) and studied by the same author in rice (Soranzo et al., 2004). Plant genomes may contain between 25 and 60 GST genes. Five different classes are recognised in plant taxa, with two of them plant specific, Phi and Tau. Their main interest for phytotechnologies is the formation of glutathione-xenobiotic conjugates, in phase II of the contaminant metabolism. Genomic approaches have led to the isolation and identification of GST genes in important species, such as Arabidopsis, rice, maize, soybean. Rice contains 61 genes, 55% of which seem to be expressed based on EST libraries. The Tau class is the most expressed. However, many GST enzymes are inducible and expressed only in the presence of specific factors. As far as genome structure is concerned, 30 GST genes cluster on chromosome 10 of rice; this feature is in common with other plant species. Evolutionary studies suggest that the plant-specific families Phi and Tau evolved in response to stress challenges by toxic compounds. Co-evolution of GST and other enzymes involved in xenobiotic metabolism, cytochromes P450 and ABC transporters, will be of great interest to understand the biochemical and physiological resources that plants can play to deal with contaminants.

2.2.2 Analysis and Identification of Enzymes and Proteins

For identification of new enzymes involved in xenobiotic metabolism proteomic approaches are also pursued. A recent example is the isolation of a new glucosyltransferase from *Arabidopsis thaliana*, responsible for the detoxification of 3,4-dichloroaniline (Loutre et al., 2003). After purification from in vitro cell cultures, the enzyme was characterised with MALDI-TOF MS and cloning of the gene was possible based on sequence information. Inducibility of the enzyme by herbicide safeners may contribute to elucidate the interactions among xenobiotics and plant metabolism.

2.2.3 Transgenic Approaches

Metabolic modification and degradation of a xenobiotic molecule may depend on a single enzyme. A transgenic approach for modifying or improving this enzyme with benefit for the relevant phytotechnology, is therefore conceivable. Examples reported in literature concern engineering herbicide tolerance, since these compounds are completely assimilable to environmental xenobiotics. For instance, Diderjean et al. (2002) reported of a successful transgenic approach with a gene for cytochrome P450, involved in Phase I of the metabolism. The gene chosen is inducible by chemical stress (metals and drugs) in Jerusalem artichoke, and it conferred resistance to phenylurea upon transfer in the sensitive species tobacco and Arabidopsis. This gene may further be considered a useful tool for phytotransformation application in case of contamination by herbicides in soils and water.

The following example is not concerned strictly phytoremediation, but rather phytomonitoring of organic compounds with a transgenic approach. The Danish company Aresa Biotechnology has developed a GM plant of *Arabidopsis thaliana* which can detect nitrogen dioxide emitted by explosives and signal this contact by changing to red color (Anonymous, 2003). The proposed application would be that of growing plants in areas affected by anti-personnel mines in order to contribute to decontamination of the site.

2.2.4 In Vitro Studies for Implementation

Cell cultures are utilized for bioassessment studies before practical application in constructed wetlands, especially in the case of complex contaminants, for which knowledge of the degradative patterns are not accurate or existing at all. Species differ in their uptake and metabolic capacities, and it has been shown that *Carex* (sedge) has negligible uptake of TNT, different from *Juncus*, *Phragmites* or *Typha* (Gerth and Hartmut, 2004).

3. IMPLEMENTATION OF KNOWLEDGE FOR APPLICATION

3.1.1 Constructed Wetlands

Application of constructed wetlands for treatment of contaminated waters is receiving growing interest in Europe. Several examples have been described in different meetings. EC has supported the implementation of a 10000 m^2 wetland in Portugal, to treat industrial effluents containing aniline, nitrobenzene and sulfanilic acid (Ramos et al., 2004). Other examples, which cannot be cited here for space limitations, can be found in the abstracts books of COST (www.gre.ac.uk/cost859/).

3.1.2 Short Rotation Coppice Forestry

Short rotation coppice is a plantation of trees, poplars or willows, which are kept for less than 15 years and produce plant biomass for several purposes in the paper and pulp industry. In particular, coppicing consists in cutting the trunk at the base at intervals of 2-3 years, and new shoots emerge from the stump. This type of forestry also represents a source of renewable energy, constituting at the same time a sink for atmospheric carbon. Utilizing plants which can take up heavy metals, consume CO_2 and produce biomass, combines forestry with phytotechnologies. Several authors are therefore studying metal uptake in willow and poplar, in order to assess the biodiversity existing among cultivars, clones and accessions. As a recent example, poplar clones were analyzed for uptake of several metals, showing how Cd, Zn, Al were taken up with high efficiency (Laureysens et al., 2004).

3.1.3 Interactions with Microorganisms

It is well known that plant-microorganisms interactions play important roles in phytoremediation. In recent years discovery of the role of endophytic bacteria in phytoremediation has led to several interesting considerations. Engineering endophytic bacteria of the species *Burkholderia cepacia* with plasmid pTOM increased degradation of toluene in yellow lupine plants (Barac et al., 2004), at the same time lowering toxicity to the plant.

3.1.4 Atmosphere Contaminants

Phytotechnologies have traditionally been limited to contaminants accessible through plant roots, either in soil and sediments or in water. However, contaminants may enter the plant also from the atmosphere, and a new application of the phytotechnologies may be the removal of pollutants from the troposphere (Morikawa et al., 2003). Nitrogen dioxide is a pollutant which may be taken up through stomata and incorporated into organic compounds. There is extensive variability among plant taxa in this regard, and a survey of about 300 species showed that the most efficient plant is Eucalyptus viminalis, 657 times more efficient than Tillandsia, the less efficient taxon. These plants could be used to assembly “green walls”, covering the vertical surfaces of building where plants are able to assimilate NO_2 in great quantities. Recently the authors have described a positive effect of NO_2 on plant biomass growth, defining it as a “plant vitalisation signal” (Morikawa et al., 2005), but this still waits for a confirmation.

4. NEW TRENDS

4.1 Natural Remediation

A new trend in use of phytotechnologies is favour encountered by the so-called “Assisted Natural Remediation”, clearly a non conventional application (Adriano et al., 2004). In Assisted Natural Remediation, amendments are added to the soil in order to accelerate natural processes of remediation. In the case of metals, amendments contribute to immobilization with complexation, adsorption, precipitation and chemical reactions: the main purpose is to lower the bioavailability of the metal, and not its total concentration. The literature reviewed by Adriano et al. (2004) includes successful examples to which Assisted Natural Remediation has been applied.

4.2 Biofortification

Studies on the interaction between plant tissues, heavy metals and/or trace elements have led to the concept of biofortification, in which plants enriched in micronutrient content are seen as an aid against malnutrition. Differently from phytoaccumulation of metals, which is considered as a risk for the food chain, biofortification of crops with specific elements may become advantageous (Welch and Graham, 2004). Fortified crops are suitable for growth on micronutrient-poor soil because their bioconcentration capacity will lead to higher content of micronutrients in edible tissues. Knowledge of mechanisms controlling metal accumulation is a prerequisite for elucidating the biochemical basis of these phenomena. Information is also requested for those antinutrients that decrease element availability: examples include phytic acid, fibres, and polyphenols.

4.3 Glucosinolates and Biofumigation

Biofumigation is a recent application of the properties of plant chemicals. In particular, several Brassicaceae are exploited in the fight towards pests and pathogens in agriculture due to the production of specific secondary metabolites called glucosinolates (Mithen, 2001). These sulfur-containing compounds have an anticarcinogenic activity in man, they contribute to the characteristic flavor of cruciferous plants, and their degradation products can deter herbivores and inhibit microorganisms. From this derives the use of Brassicaceae as “green manure” to be added to the soil during preparation, in order to decrease the load due to pathogens and pests. This is a sustainable substitute to the use of chemical fumigants. An interesting feature still to be explored is the possible connection between production of specific glucosinolates in the plant and the presence of heavy metals in the environment which may act as inducers or repressors. Since glucosinolates contain sulfur, like metallothioneins and phytochelatins, they could have an impact in the sulfur metabolism of these heavy metal sequestering peptides.

This in turn may determine an interlock during the process of pest resistance and heavy metal resistance.

5. CONCLUSIONS

Research on phytoremediation and phytotechnologies is thriving in Europe even despite lack of funding. Few EC funded projects and different National or regional activities has prevented research in this field to collapse. Networking activities such as those promoted within COST are also of extreme importance for building interactions and collaboration among scientists within and outside Europe. Other initiatives targeted at dissemination, education and training, should be activated in order to increase the familiarity and confidence of the public opinion and of stakeholders in these new sustainable technologies.

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CONSTRUCTED WETLANDS AND THEIR PERFORMANCE FOR TREATMENT OF WATER CONTAMINATED WITH ARSENIC AND HEAVY METALS

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Abstract: This study investigated the removal mechanisms of arsenic and heavy metals in constructed wetland systems. The biotic and abiotic processes in the wetlands and the influences of plants, soil and micro-organisms on arsenic and heavy metal removal were examined. Various small-scale constructed wetlands were set up in order to study and compare the removal efficiency of laboratory-scale wetland models and small-scale field test systems. In the field test systems, acid mine drainage (AMD) was used as an example of acidic wastewater contaminated with heavy metals.

Key words: heavy metals, arsenic, biotic – abiotic processes, constructed wetlands, rhizosphere

1. INTRODUCTION

Acid mine drainage (AMD) poses worldwide environmental problems of large dimensions wherever mining occurs. Moreover, arsenic wastes are also released by many other industries, such as the chemical and electronic

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industry, which use arsenic as a material in their processes; this implies increasing environmental problems worldwide correlated to human health (Le, 2001; Williams et al., 1996).

In general, contaminated mine water is generated when rock containing sulphidic minerals is exposed to water and oxygen. This results in the production of acidity and elevated concentrations of metals and sulphate in the water (Braun et al., 2001). The generation of contaminated mine water is a combined chemical and microbiological process (Stumm and Morgan, 1996). Factors influencing the acidity and metal content are the sulphide grain size and surface area, porosity and permeability of the deposit, nature of the gangue materials, nature of the sulphide ore, nature of acid-consuming minerals, and various environmental factors influencing the activity of micro-organisms (Ritcey, 1989). The generation of the water is highly site-specific: it can vary greatly even within a single mine site and the chemical composition may be very different including elements such as Al, As, Cd, Cu, Fe, Pb, Mn, Ni and Zn with higher or lower concentrations of sulphate and pH-values ranging from 2.6 to 7.5 (Ritcey, 1989; Wildeman and Laudon, 1989; Dodds-Smith et al., 1995).

The primary aims of the treatment of contaminated mine water are to neutralize acidity and to remove metals (Braun et al., 2001). In principle, two broad categories of available types of treatment can be used: (1) active systems, mainly for continuous operation and maintenance and (2) passive systems, which are intended to be self-sustaining after an initial start-up period (Braun et al., 2001). Active systems involve technologies such as pH modification, ion exchange, biology-based treatments, adsorption, electrochemical treatment and physical process technologies. Passive treatment includes chemical passive treatment by the addition of chemicals such as limestone, polymers or others (Braun et al., 2001; Brodie et al., 1993; Ziemkiewicz et al., 1997) and biological passive treatment represented by primary constructed wetlands and secondary algal systems or special bioreactors (Braun et al., 2001; Foster, 1982; Phillips et al., 1994; Bender et al., 1994; Davison, 1993; Duc et al., 1998). The passive treatment systems, and in particular constructed wetlands, are advantageous mainly because they cause comparably low costs, are truly self-sustaining and are suitable for the treatment of mine water from abandoned mines (Braun et al., 2001).

The aqueous chemistry of arsenic is very complex because arsenic can exist in solution in the -3, +3 and +5 oxidation states. The predominant aquatic forms are the trivalent arsenite and the pentavalent arsenate. Arsenates are less soluble and therefore less toxic. Arsenate is predominant in oxic surface waters, whereas arsenite is present in anoxic waters such as groundwaters. The oxidation from the arsenite to the arsenate state by oxygen proceeds at room temperature only with the aid of catalysts. In an extended literature review by Riveros and Dutrizac (2000), the chemistry of arsenic is described in detail with the aim of deriving recommendations for achieving long-term stability of arsenic wastes.

Nishimura et al. (1996) studied the oxidation of arsenite by oxygen in presence of sulphite and Fe(II). The authors conclude that the oxidation reactions of S(IV), As(III) and Fe(II) induce each other and result in a fast As oxidation and precipitation by Fe(III). The structure of the Fe(III)-As(V) precipitate according to the formation equation

$$Fe^{3+} + AsO_4{}^{3-} + x\ H_2O \rightarrow FeAsO_4 \,.\, xH_2O \qquad (1)$$

is controversial. Robins et al. (1991) and Robins and Jayaweera (1992) demonstrated that the precipitate is similar to a poorly crystalline ferric oxyhydroxide phase (2-line or 6-line ferrihydrite) with adsorbed arsenate ions. As(V) is adsorbed on ferrihydrite by complexation, resulting in a stable inner-sphere complex. Such inner-sphere complexes are extremely stable against desorption.

In waterlogged soils, bacterial activity may include in this reaction chain sulphur(VI)-reduction and formation of iron(II) in anaerobic zones. In soil with a high content of organic matter, the complex link between biotic and abiotic reactions will be made nearly unforeseeable by the interaction of humins as alternative electron acceptors. In the case of wetlands, the presence of plants will further increase the complexity of this multi-component system up to a level at which the system can only be regarded as a "black box". The scheme in Figure 1 is an attempt to summarise some of the reactions, according to the scheme of Bhumbla and Keefer (1994).

Constructed wetlands have been used for mine water treatment for about 20 years. However, the potential longevity of constructed wetlands for mine water treatment is currently not known and the design concepts and sizing criteria are still under discussion (Braun et al., 2001).

The removal mechanisms in wetlands may include metal incorporation/accumulation into below- and above-ground plant tissues, adsorption on soil components, filtration/sedimentation of suspended particles and microbial actions causing precipitation and co-precipitation and/or volatilisation in the cases of certain metalloids etc. (Matagi et al., 1998; Jackob and Otte, 2003; Dushenko et al., 1995).

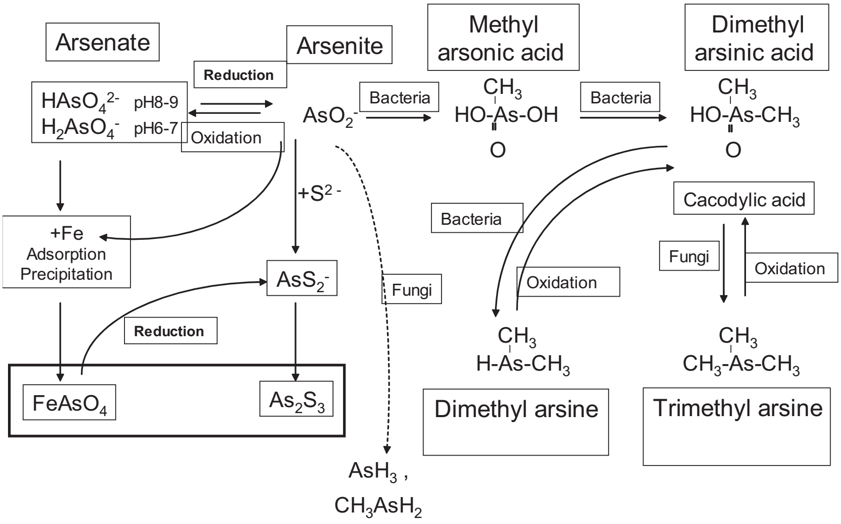

Figure 1. Chemical forms of As and their transformations (modified from Bhumbla and Keefer, 1994).

Wetland plants are able to accumulate arsenic and heavy metals, whereby the content in the roots is considerably higher in comparison to the above-ground plant matter (Stoltz and Greger, 2002). Nevertheless this metal uptake and accumulation by plants plays only a minor role in wetlands for water treatment (Mays and Edward, 2001). As and Zn can also be bound to iron oxides as a consequence of oxidation and the formation of so-called iron plaque on the roots of wetland plants (Doyle and Otte, 1997, Mendelssohn et al., 1995). The presence of anoxic and oxic zones with gradients of redox potential in the wetland system seems to be one of the main pre-conditions for the functioning of all the above-mentioned biotic-abiotic reactions. This redox potential will be influenced in general by the concentration of the ionic redox elements, the pH-value and the oxygen concentration. Some places of gradient formation are listed in Table 1.

The goal of the study was to characterize the fate of arsenic, zinc and iron in various wetland systems with different conditions for water flow and gradient formation. The removal of the toxic elements should be optimised in laboratory systems and in small-scale field systems with acid drainage waters.

Table 1. Main gradients in a subsurface-flow constructed wetland.

Gradients of	Sphere
Eh:	soil organic matter
	pore water
	rhizosphere
	biofilms on the root surface
	biofilms on the soil (gravel) particles
O_2:	shoots (aerenchyma)
	roots (aerenchyma)
	biofilms on the root surface
	biofilms on the soil (gravel) surface
pH:	pore water
	water
	biofilms

2. MATERIAL AND METHODS

2.1 Laboratory-Scale System of Wetlands and Ponds

A more detailed description of the experimental system can be found in Buddhawong et al. (2005). The model wetlands were set up in plastic containers (0.5 m long, 0.3 m wide and 0.3 m high) each with a total volume of about 45 litres and a surface area of 0.15 m^2.

In the *Subsurface Water Wetland* (SWW) system, plants of *Juncus effusus* were planted in a gravel bed (grain size of 0.2 – 0.6 cm). The height of the gravel bed was 25 cm, and the water level was kept 5 cm below the gravel bed surface whereby the pore water volume was about 14 litres.

In the *Free Surface Water Wetland* (FSW) system *Juncus effusus* was planted in the same container type and same gravel material as for the SWW. However, the height of the gravel layer was only 15 cm and the water level was kept 10 cm above the gravel layer, resulting in a total (free and pore) water volume of about 25.5 litres.

The *Algae Pond* (AP) was in the same container type as for SWW and FSW but without gravel. The water level was at about 22 cm height (33 litres). With time, algae growth started without additional inoculation.

A glass column of 0.3 m diameter with 0.07 m^2 surface area was used as a *Hydroponic System* (HP). The water level was at 0.6 m height; the water volume was 41.5 litres. The floating *Juncus effusus* plants covered the whole surface area.

The *Juncus effusus* plants used were propagated in hydroponic culture in a greenhouse. The plants were transferred into the model systems three month before starting the experiments.

The experimental systems were operated in a greenhouse; the temperature was adjusted to 25 °C. At the beginning of the experiments the total plant biomass (roots and shoots; in kg wet weight) was 4.2 for SSW, 1.7 for FSW and 1.3 for HP. Within the experimental period of 90 days (16 July – 15 October 2001) the biomass increased by 10.8%, 76.0% and 30.3% respectively.

The model water was prepared by using tap water containing the following main components (in mg/L): Ca 90.7, Mg 12.6, K 4.6, Na 23.0, SO_4^{2-} 157, Cl^- 45.5) to which the chemicals were added according to Kuschk (1991). Furthermore $ZnSO_4$ and As_2O_5 were added, resulting in a final concentration of 5 mg/L for Zn and 0.5 mg/L for As. Finally, the artificial wastewater was adjusted to pH 4 by adding H_2SO_4.

Because of evapotranspiration, each container was topped-up with distilled water twice a week in order to maintain the water level in all containers at the start-up level.

Sulphate was measured by means of ion chromatograph using a Dionex 100 (AS4A-SC column/AG4A-SC column) with conductivity detection (Rethmeier et al., 1997)

Water samples were taken from 3 different levels of the model systems: near the bottom (about 3 to 5 cm above the bottom), in the middle (e.g. in the middle between bottom and the surface of the respective gravel beds or in the middle of the water column for HP) and near the surface (about 10 cm below the surface of the gravel bed for SWW and about 5 cm below the water surface of the other experimental systems).

2.2 Small-Scale Field Test Systems for AMD Treatment

A more detailed description of the experimental system can be found in Buddhawong et al. (2005). The model experiments were conducted at the UFZ experimental area in Grosskayna-Beuna, near Merseburg (Germany). These field test systems consisted of 6 small containers, which were continuously fed with acid mine drainage (AMD) from the "Merseburg-Ost" gravel pit. This AMD had a concentration of 2-3 g/L sulphate and its pH was about 3. The further main chemical elements of this AMD were (in mg/L): Na ~450, Ca 390, Fe 150, Mg 120, Al 90, Mn 9, K 3, Zn 1.8, Ni 1.3, Br 0.9, Li 0.7, Co 0.6 and Ce 0.5; further elements were also detectable at lower concentrations.

The 6 different model constructed wetlands were each placed in containers with a size of 0.47 × 0.60 × 0.91 m^3 and a surface area of 0.55 m^2. Further parameters are summarized in Table 2. *Juncus effusus* was used in all the planted wetland systems. Container B1 and B2 represented the hydroponic systems (HP). FSW and SSW (B3, B4, B5 and B6) contained a mixture of sand materials, which included sand and fine gravel (0.6-2 mm size). The water was kept flowing 10 cm below the sand surface of SSW, and 10 cm over the sand surface of FSW. The inflow rate in different systems differed from 0.1 -0.4 l/h, the hydraulic retention time 8 and 40 days. For details see Buddhawong (2004).

2.3 Analytical Methods

The redox potential was measured with a SenTix ORP electrode connected to a Multiline P4 (WTW, Germany). To prevent air contact the electrode was put into a small flow-through cuvette. The inlet of the cuvette was connected with a long robust injection needle which was pushed into the required depth of the model wetland/pond systems. The outlet of the cuvette was connected with a syringe to suck water samples through the cuvette containing the electrode. All data were recalculated to the standard hydrogen electrode in accordance with the temperature.

As, Zn and Fe were analysed by ICP-AES (Spectro Cieros) (APHA, AWWA, and WEF, 1995). The detection limits were 0.06 mg/L for As, 0.04 mg/L for Zn and 0.05 mg/L for Fe, whereby the analysis of triplicates was found to be in all cases within a standard deviation of ± 5 %.

3. RESULTS AND DISCUSSION

3.1 Removal Performance of Arsenic and Zinc in the Laboratory-Scale Wetlands

3.1.1 Arsenic

The arsenic concentration gradients along the sampling levels (bottom, middle, surface) of the 4 experimental systems are shown in Figure 2. In SSW and FSW, in all cases the concentration decreased after 24 days to below 0.1 mg/L. However, a no significant decrease of arsenic occurred in AP and HP.

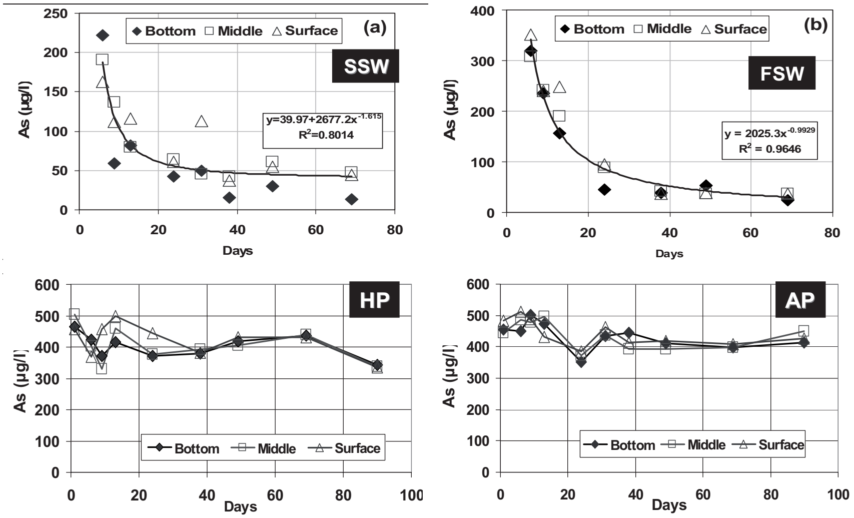

Figure 2. Arsenic concentration in the water of the laboratory model wetlands, dependent on the time and sampling depth.

In unplanted gravel vessels, a very low adsorption capacity (about 4 µg/kg gravel) was measured. Therefore we conclude that there was no significant direct sorption to the matrix. With the same systems, the adsorption or incorporation of arsenic into the plants was determined. However, this amount of arsenic was also found to be negligible (Buddhawong et al., 2005). As a consequence, the explanation for the phenomenon of greatest arsenic removal in the planted gravel system could only be that gravel in combination with plants provides especially favourable conditions for arsenic binding.

It was of further interest to determine the concentration of iron, the behaviour of pH and the redox value with respect to the factors influencing the complex system.

The pH value decreased to about 4 in HP; however, in SSW and FSW the pH remained relatively constant between 6 and 7 (data not shown). The redox potential of the SSW and the FSW in dependence on the sampling level is shown in Figure 3. The redox value was lowest at the bottom of both systems. However, in the other systems (AP and HP) no differences in the redox values were observed (data not shown).

It can be assumed that due to the activity of the roots, organic compounds (rhizodeposition products as the sum of root exudates and dead root matter) are released into the rhizosphere. Some of these compounds can function as iron chelating compounds (Hoffland et al., 1992). Furthermore, these organic

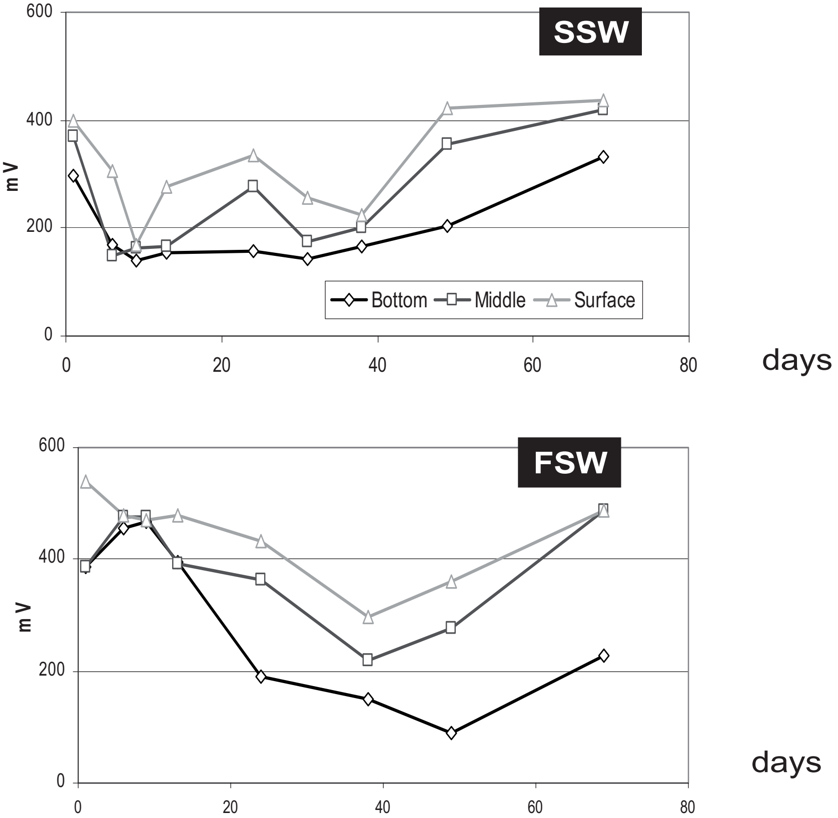

Figure 3. Redox potential in the water of the laboratory model wetlands, dependent on the time and sampling depth.

compounds can also be used as a carbon source for micro-organisms in the soil, resulting a decrease of the redox potential. Both the relatively low redox (see Figure 3) and the chelating rhizodeposition products stimulate the redissolution of crystalline iron(III), which has a low binding capacity for As. Nevertheless, because of the capability of some helophytes to transfer oxygen into their rhizosphere (Jackson and Armstrong, 1999; Colmer, 2003) the oxic conditions in the rhizosphere and especially on the rhizoplane can cause the precipitation of the dissolved iron and co-precipitation of other trace elements such arsenic, especially on the roots forming iron plaques (Wang and Peverly, 1996).

The time course of the formation of dissolved iron and the subsequent decrease of iron concentration in the pore water especially at the bottom of SWW (see Figure 4) supports the assumption that, in general, a dissolution of crystalline iron and subsequently precipitation by the direct and indirect action of plants and micro-organisms in combination can cause the observed As removal from the water phase in waterlogged soils having an apparently low As binding capacity.

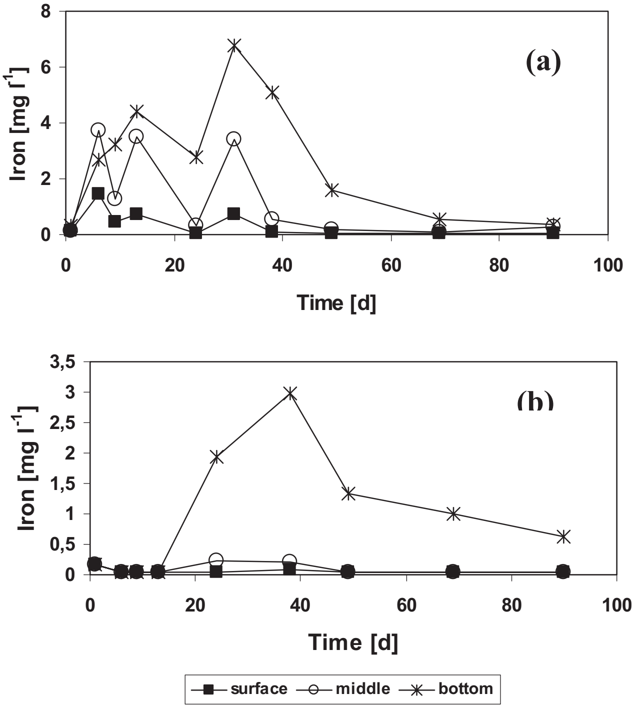

Figure 4. Iron concentration in the water of the laboratory model wetland (SWW), dependent on the time and sampling depth (a: SSW, b: FSW).

3.1.2 Zinc

The aqueous chemistry of zinc is simpler than that of iron and arsenic. Only the formation of insoluble sulphide or carbonate at neutral or higher pH could be responsible for Zn elimination from solution. In our experiments, the zinc concentration only decreased in SWW, FSW and HP. In AP, the Zn concentration did not change significantly during the period of 90 days. Almost complete elimination was observed for both the planted gravel bed systems, whereby in SWW the rate was faster than in FSW (see Figure 5).

In HP, the Zn concentration decreased only slowly by about 27 % during 90 days (data not shown). Similar to the total As concentration, no concentration gradient dependence on the sampling depth could be observed.

Because of the extremely fast decrease of Zn concentration in both gravel bed systems (SWW and FSW) within few days, the increase of iron concentration with time (see Figure 5) and the observation that the redox potential did not decrease significantly below 100 mV (see Figure 3), the action of sulphate-reducing bacteria and resulting sulphide precipitation can be excluded.

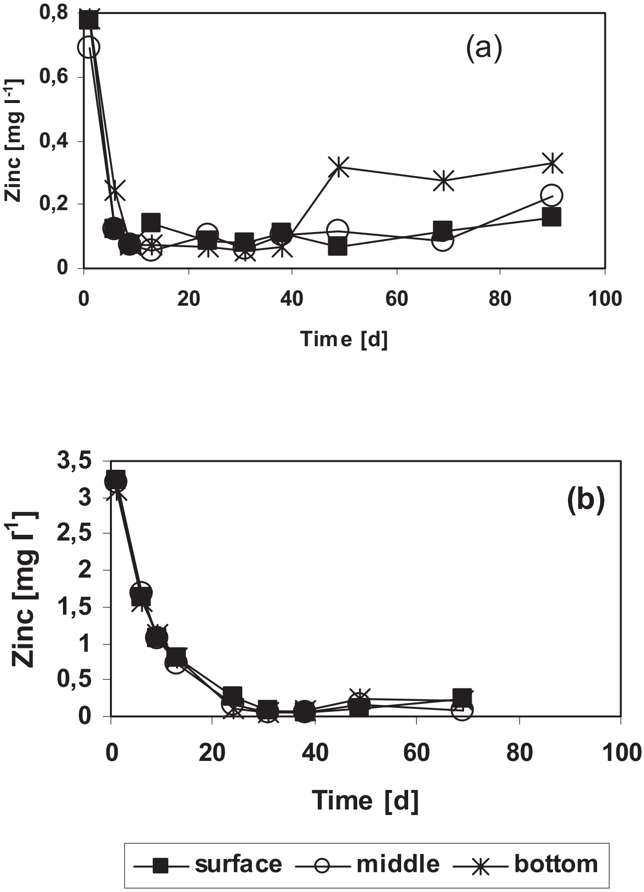

Figure 5. Zinc concentration in the water of the laboratory model wetlands, dependence on the time and sampling depth (a – SWW, b – FSW) (from Buddhawong et al., 2005).

Whether Zn is sequestered as its carbonate within the gravel systems because of higher pH values in comparison to HP and AP is still an open question.

3.2 Treatment of Acid Mine Drainage (AMD) in Small-Scale Constructed Wetlands in a Field Experiment (Preliminary Results)

Operating in an open area, six field test systems were fed continuously with an AMD of pH 3 in long-term experiments from spring to wintertime. The results are summarized in Figure 6. As in the previous experiments

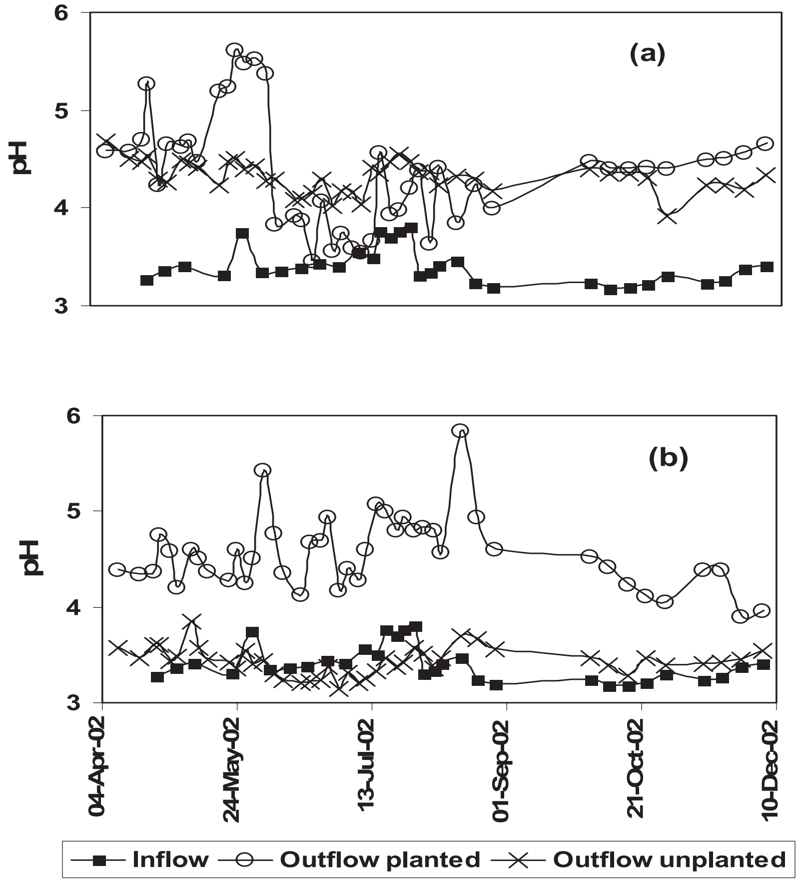

Figure 6. Changes of pH over time of the AMD in the various small-scale constructed wetlands in the field test.

under batch conditions, the SSW and FSW with gravel beds show a clear pH increase at the outflow in comparison to the inflow AMD. However, when passing through the HP the pH of the AMD remained almost unchanged.

The behaviour of iron and zinc in this continuous flow system is being studied as part of our current work.

4. CONCLUSIONS

The removal capacities for As and Zn are larger in soil- (gravel-) based wetlands than in hydroponic systems or in algae ponds.

Probably due to diffusion limitations, depth-gradients were found in the soil-based systems according to various parameters of the pore water such as redox potential and concentrations of contaminants. These gradients appear to be a prerequisite for the complex iron dissolution and re-precipitation processes within the soil-based systems.

Although gravel and plants each separately have low binding capacities for As, it was found that the combination of both within a single system obviously effects a dissolution of soil crystalline iron to act as a co-precipitation agent for As, particularly in the oxic zones of the rhizoplane.

The small-scale experiments simulating the treatment of acid mine drainage by various types of constructed wetlands operated on a field site showed, in general, similar results. Wetland systems with a combination of gravel/soil matrix and plants have a better treatment performance than systems with only soil or only plants without soil.

In the field experiments, the seasonal change, rainfall and temperature affected the removal efficiency of the constructed wetlands.

In general, these results encourage the implementation of constructed wetlands for remediation of wastewater from mines and industries which emit acidity, As, Zn and other heavy metals.

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DISPOSAL OF SEWAGE EFFLUENT AND BIOSOLIDS IN EUCALYPTUS PLANTATIONS: A LYSIMETER SIMULATION STUDY

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Abstract: Reduced soil leaching under nutrient-rich sewage effluent irrigation and biosolids compost application was tested as means to control leaching of nutrients, organic carbon, heavy metals and enteric bacteria. Two-hundred liters, sand-packed lysimeters were either applied with biosolids compost (at rates equivalent to 125 and 625 Mg/ha) or not applied, and 3-times daily surface drip irrigated with either oxidation ponds effluents or fertilized fresh water. Irrigation peaked at nearly 60 L/day·lysimeter. Lysimeters were planted with a *Eucalyptus camaldulensis* tree, and leaching of planted lysimeters was either intermittent or at leaching fraction (LF) of 0.2. Without a tree the LF was near 1. Results from the 2nd year of the experiment are presented. The trees resisted the increasing salinization of the soil solution caused by the combination of low LF and frequent irrigation. Applied nitrogen was nearly completely intercepted in the soil-tree system, which was less efficient with respect to phosphorus. The fate of P in the studied system was governed by uptake and precipitation, the latter probably being retarded by low Ca and Mg activities (despite their overall high concentrations). Leaching of heavy metals was minimal even at the heavy biosolids application rate. Counts of fecal coliforms in the leachates were most often nil under LF 1, yet they were substantial under low leaching. Still, the overall recoveries of the bacteria in the leachate corresponded to the LF in a bell-shaped pattern, with maximum recoveries of 5-40% at LF 0.2. The main parameter that positively correlated with this recovery pattern was the leachate BOD. Hence, the simulation study showed that inasmuch as the trees withstood elevated salinity levels and the soil-plant system effectively intercepted organic and inorganic pollutants and bacteria, yet its long term sustainability (in terms of salinization and sodicity) should not be overlooked.

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Key words: Biosolids; effluent irrigation; enteric bacteria; *Eucalyptus*; fertigation, heavy metals; leaching; lysimeters; nitrogen; organic carbon; phosphorus; salinity

Abbreviations: BC - biosolids compost; BOD - biochemical oxygen demand; DOC - dissolved organic carbon; FC - fecal coliforms; ET - evapotranspiration; LF - leaching fraction (leaching as percent or fraction of amount of irrigation water); OC - organic carbon; PET - potential evapotranspiration; RT - residence time.

1. INTRODUCTION

The paper focuses on disposal of wastewater in small communities, generally towns of <20,000 population, especially, but not only, in less developed countries. Currently, the uncontrolled disposal of low-grade effluent and biosolids might result in pollution of rivers, soil and ground water, and be a source for emissions of greenhouse gases (IPPC, 1997). We perceive recycling of wastewater products through agriculture and forestry a viable and feasible option, which promotes pathways of aerobic degradation of organic matter and the assimilation of degradation products in plant and in stabilized soil humus. Thus, recycling minimizes emissions of greenhouse gases and leaching and run-off of nutrient elements, trace and heavy metals, organic matter, enteric microorganisms and salinity components (Feigin et al., 1991; Pescod, 1992; Stentiford et al., 2004). Because the rates of uptake and/or degradation of these constituents in the soil-plant system are often slower than their loading ratio in the soil, sewage effluent irrigated and/or sludge amended fields can become a source for organic and inorganic contaminants (Ongley, 1996). In this respect, nitrate leaching and pollution is a primary concern (see e.g., Steenvoorden and Endreny, 2004).

Irrigation with effluent and sludge addition to soils can adversely affect soil and groundwater. These sewage products add organic carbon (OC) to the soil, part of which is dissolved organic carbon (DOC) (Fine et al., 2002a). Effluent DOC can influence soil properties and behavior (e.g., soil redox potential, Patrick and Jugsujinda, 1992), and can interact with microcontaminants to enhance their transport in the soil, the unsaturated zone and the groundwater (Gerba et al., 1975; Frankenberger, 1985; Fine et al., 2005; Fine et al., 2006). Irrigation with low-grade sewage effluent in the central Mexico valley greatly increased the contents of total OC and DOC in the upper soil layers, and enhanced metal solubility and transport down the soil profile (Siebe and Fischer, 1996). They suggested that the rate of migration of heavy metals was enhanced by the reductive dissolution of manganese oxides in the soil. Application of anaerobically digested, waste-activated sludge to soil was also shown to increase the solubility and migration of trace elements (Lamy et al., 1993; Fine et al., 1997; Vulkan et al., 2000). Effluent irrigation also substantially enhanced the transport of organic micropollutants, which were applied to field soil at agronomic rates and as regular practice (Graber et al., 1995). Seol and Lee (2000) suggested that slight but persistent suppression of sorption of pesticides, together with alteration of other properties of the soil solution by effluent water components, could be important in the mobilization of pesticide in soils.

The disposal of sewage effluent for forest plantation irrigation has become wide spread worldwide with tree benefiting from the nutrient and water supplies (Mitchell and Ford-Robertson, 1992; Myers and Lehane 1999). *Eucalyptus camaldulensis* is a genetically variegated tree species that is widely distributed, and is very adapted to saline environments (Sun and Dickinson 1993; Zohar and Schiller, 1998). Canopy N and P contents of a 41 months old *E. camaldulensis* stand were 204-240 kg N/ha and 10.4-12.2 kgP/ha at 833 and 2220 trees/ha, all respectively (Harrison et al., 2000). Frequent harvesting of trees enhanced nutrient uptake from the soil (Guo et al., 2002).

We used a lysimeter setup to forecast the efficacy of the soil – tree system in intercepting nutrients, heavy and trace elements, organic carbon and enteric microorganisms applied to soil in secondary effluent irrigation and BC.

2. MATERIALS AND METHODS

2.1 Lysimeter Construction, Treatments and Water Residence Time

Data were collected in a lysimeter setup, the design of which is presented in detail elsewhere (Fine et al., 2002a). Briefly, it consisted of 0.7-m deep, 200-L sand packed drums that were either planted with a *Eucalyptus camaldulensis* tree, or not planted. *Eucalyptus* was chosen for the study because it is fast growing and resistant to both water logging and drought and to salinity. It is common in ornamental and commercial planting in Israel.

Irrigation was three times daily, by eight surface drippers per lysimeter. Fertilized fresh water or low-grade secondary effluent was used; the latter was pumped from an adjacent oxidation pond (Table 1). The fertilizer solution contained trace elements (Fe, Mn, Zn and Cu) chelated with EDTA. Sludge compost (Table 1) at 0, 50 and 250 g/kg was mixed in the upper 0.2-m layer (equivalent to 0, 125 and 625 Mg/ha). Irrigation water volumes changed according to the evapotranspiration (ET) and the required leaching. Planted lysimeters were tested at leaching fraction LF 0.2 or at intermittent leaching; each in 6 replicates. Not planted lysimeters received same amounts of water as the planted ones (in 2 replicates; one pair for each leaching fraction), which provided 90-100% leaching. The treatments and the designated and actual leaching fractions (LFs) are presented in Table 2.

Table 1. Composition of the irrigation water and biosolids compost.

Parameter, Component	Units	Effluent	Fresh water	Biosolids compost (BC)
pH		7.76	7.4	
EC25	dS/m	1.96	1.40	
OC	mg/L	192	7.84	210,000
BOD	mg/L	134		
Total bacteria	cfu/ml	106.9		
Total coliforms	cfu 100/mL	105.9		
Fecal coliforms (FC)	cfu 100/mL	105.3		
Fecal Streptococci	cfu 100/mL	103.8		
B	μg/L	590	150	25
Ba	μg/L	50	47.3	527
Ca	mg/L	92	56	90,000
Cd	μg/L	0.3	0	6.4
Cl	mmolc/L	10.4	6.5	
Cu	μg/L	45	28	633
Fe	μg/L	454	248	14,440
K	mg/L	41	28.5	3,184
Mg	mg/L	36	30.5	8,447
Mn	μg/L	72	127	241
N Kjeldhal	mg/L	54	25.6 (as NH_4)	14,900
Na	mg/L	270	119	2,700
Ni	μg/L	29.4	8.8	81
P	mg/L	17	14	15,400
Pb	μg/L	5	<3	136
Zn	μg/L	166	260	2,468

Table 2. Designated and actual leaching fractions in the lysimeter experiment, water uptake of the *Eucalyptus camaldulensis* trees, irrigation heads and canopy yields. Data from the 2nd year is presented[1] (values are means and standard deviations).

Treatment parameters			Actual LF (%) (Aug 1 – Dec 5)	Daily ET[3] (L/tree): (Aug 1 – Dec 5)	Overall ET[4] (m^3/tree) (Aug 1 – Dec 5)	Season irrigation head[5] (m) a)per soil b)per plant	Canopy yield (kg dry wt/ tree)[6]
Water type	Bio-solids compost (Mg/ha)	Designated LF (%)[2]					
Effluent	All[7]	100	97.5 ±0.75				
	0	20	21.5±8.3	25.6±3.1	5.37	a) 30.9 b) 1.06	15.1 ±2.9 ab
	0	Intermittent leaching	10.6±4.7	18.6±3.2	2.80	a) 23.7 b) 0.94	16.3 ±4.7 a
	125	20	17.1±6.3	19.1 ±12.2	3.80	a) 31.0 b) 1.12	14.2 ±0.7 ab
	125	Intermittent leaching	8.5±1.8	16.7±2.0	2.52	a) 23.3 b) 0.94	17.9 ±3.0 a
	625	20	23.6±1.6	19.6±8.8	2.97	a) 26.8 b) 0.90	12.1 ±0.9 ab
	625	Intermittent leaching	10.3±2.1	14.9±3.1	2.26	a) 20.7 b) 0.82	9.4 ±2.2 b
Fresh	All[7]	100	97.5±0.1				
	0	20	21.2±8.3	28.1±2.4	4.24	a) 27.0 b) 0.91	15.1±2.2 ab
	125	20	22.2 ±10.7	25.1±3.5	3.79	a) 28.0 b) 0.87	18.8±3.1 a
	625	20	18.4±7.1	21.7±3.7	3.28	a) 24.8 b) 0.86	15.7±4.1 ab

1) Irrigation season was 257 days, March 23 through Dec. 5.

2) Irrigation at LF 0.2 was conducted for the whole 257-day period. Intermittent leaching deviated from LF 0.2 on August 1 and maintained for the remaining 126 d.

3) Daily ET was calculated every other day throughout the period – Aug. 1st- Dec. 5th, based on measured irrigation and leachate volumes. During the first period it was monitored less often.

4) Overall water uptake per treatment is the cumulative water uptake by the tree in each lysimeter during the relevant period. These amounts were averaged for each treatment.

5) The irrigation head (ET + leaching) is based on the cumulative volumes of water applied to each lysimeter during the entire irrigation season (257 days). Parameter (a) shows the effective leaching of the soil in the lysimeter, according to the 0.25 m^2 cross section of the drum. Parameter (b) shows the equivalent irrigation of a commercial plantation with

planting density of 1660 trees/ha (6 m^2/tree). The conversion factor between the two parameters is 24 (6/0.25).

6) Means followed by the same letter and not significantly different. Tukey's Studentized Range (HSD) Test was used at $\alpha < 0.05$ by the GLM procedure by SAS.

7) Average of all the not planted lysimeters, effluent and fresh water irrigated together. Note, that each planted treatment had its not-planted counterpart.

The lysimeters were planted in October 1994, and data from the second year of the experiment is presented (1996). Irrigation monitoring started on March 23 of this year and continued until December 5 (with no significant rains in this period). The intermittent leaching treatment began in August 1, by re-adjusting the water head in lysimeters that were formerly irrigated at LF 0.2.

The predesignated leaching fraction was maintained by frequent (every other day during the second half of the season) monitoring the amount of leachate for each lysimeter. The subsequent water heads were then corrected if so needed, to mach the measured evapotranspiratopn (ET) (with reference to forecasted potential evapotranspiration PET in the next interval), with/without the assigned excess for leaching. To accommodate for differences between trees in the same treatment, individual water heads were corrected by omitting drippers.

A mean seasonal residence time (RT) of the water within the soil profile was calculated for each lysimeter separately, and averaged for each treatment. The RT in the treatments presented here ranged from >20 days to <1 day depending on the LF and the water uptake (Fine et al., 2002a). Irrigation heads were calculated in two ways: (i) by dividing the overall amount of irrigation water by the soil surface area (0.25 m^2, the cross section of the lysimeter). This provides an equivalent of amount of water that was available for soil purging at the soil surface. (ii) by multiplying the cumulative amount of irrigation water per tree by 1660 (6 m^2/tree), which is common planting density in commercial plantations. The conversion factor between the soil leaching factor and the tree irrigation factor is 24 (6 m^2/tree 0.25/m^2). This means that soil leaching in the lysimeter was 24 times more intense than in a commercial plantation, at same irrigation head.

2.2 Water and Soil Analyses

Leachate samples were collected in 2-4 weeks intervals under confined atmosphere. Trace elements were determined by ICP-AES ("Spectro-flame Modula E" from Spectro GMBH, Kleve, Germany), either directly or following destruction of organic matter and volume reduction by boiling at 2% HNO_3 concentration. Precipitation was avoided by minimizing the volume reduction, and if appeared it was either dissolved by dilution or the process was repeated. The BOD_5 test was done using method 5210-B of the APHA (Clesceri et al., 1989). Dissolved carbon forms were analyzed using a DC-190 high temperature TOC analyzer (Rosemount Analytical, CA). NH_4 and NO_3 were measured colorimetrically with a Lachat autoanalyzer, Milwaukee, WI). Chloride was measured using a Jenway chloridometer (model PCLM3)

2.3 Fecal Bacteria Essay

Fecal bacteria were analyses in effluent and leachate samples on the day of collection. Leachate bottles were in ice boxes during collection. Membrane filtration (MF) was used to collect the microorganisms according to Clesceri et al. (1989) part 9222B. Fecal coliforms (FC) and Fecal streptococci (FS) were determined using methods in parts 9222D and 9230C, respectively. Results are expressed as colony forming units (cfu) per 1 or 100 mL.

2.4 Statistical Analtsis

Analyses used SAS PROC GLM (SAS 9.0, SAS Inst. Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1 Tree Growth, Water Uptake and Soil Leaching

Average dry weight of the 2nd year canopy regrowth (following cutting at the end of the 1st year) ranged from 9.4 to 18.8 kg/tree. Under effluent irrigation, all of the trees survived the cutting, and their regrowth seemed not to respond to either the reduced leaching regime or to the lower biosolids compost BC addition rate (125 Mg/ha). The high BC application rate, at 625 Mg/ha, seemed to retard tree growth, especially under intermittent soil leaching. The only significant difference in tree weight between treatments was two between intermittently leached effluent irrigated treatments: no BC added and 625 Mg BC/ha

However, the BC treatments caused more growth retardation in combination with fresh water fertigation than with effluent irrigation. Thus, while 42% of the trees in the former combination did not recover the first year cutting, no tree has died in the latter combination (or to that sense, in any of the other treatments). Interestingly, the growth of the trees that survived the first year cutting in the fresh water fertigation – BC combinations was in the higher range of tree weights in the experiment. This phenomenon will be more elaborated on below, where we deal with the role of EDTA in the system.

The mean daily water uptake of the effluent-irrigated trees at LF 0.2 peaked at near 45 liters/tree·day^{-} (Figure 1). Generally, the water uptake pattern followed the PET. Shifting trees to intermittent leaching on August 1 immediately decreased their water uptake, most probably due to increased soil salinity (below). The mean daily water uptake of the trees in the second part of the season (Aug 1st – Dec 5th) was 19-22 and 23-30 liters/tree·day under intermittent leaching and LF 0.2 regimes, respectively. Similar transpiration rates of 38.5 liters/tree·day were measured in the midst of hot and dry Israeli summer for 3 years old *E. camaldulensis* trees (1300 trees/ha) that grew on top of a brackish (2-21 dS/m) shallow water table (Zohar and Schiller, 1998).

Based on the daily mean water uptake of the trees, in the first (not shown) and second parts of the season (Aug 1st – Dec 5th), we estimated that the water uptake (ET only) of a 2-year old, commercial *Eucalyptus camaldulensis* plantation at planting density of 1660 trees/ha (a 6 m^2/tree planting grid) is approximately 1-m/yr or 10^4 m^3/ha·yr (Table 2). Using the same data and calculating for the lysimeter cross section (0.25 m^2) we also calculated the effective water head was at 21-31 m/yr (Table 2), which intensely purged the soil surface (and profile).

Hence, the young *Eucalyptus* trees produced substantial canopies to transpire large volumes of water. The mean transpiration rate of the 2-year old trees, from Aug 1 to Dec. 5, was 15-28 liters/tree·day.

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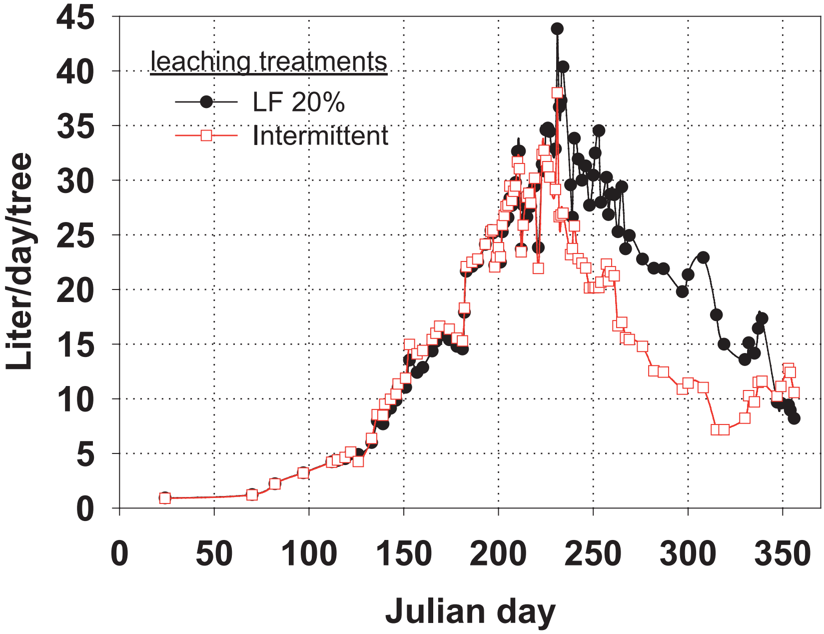

Figure 1. Average daily evapotranspiration of *Eucalyptus camaldulensis* trees grown in sand-packed lysimeters as affected by leaching treatment. Irrigation was 3 times daily, every day. The data is from the 2nd year of the study.

3.2 Leaching of Applied Nitrogen

Data for nitrogen leaching is presented in Figure 2 for sand-packed lysimeters with BC (applied at load equivalent to 625 Mg/ha) or without BC application, under irrigation with effluent or fresh water at LF 1 (no tree) or LF 0.2 or under intermittent leaching. While the entire N in the effluent was ammonium and organic N (Table 1), practically all of the N in the leachates was nitrate, with mere traces of the precursors. The lysimeters were sampled in 2-4 week intervals from March to December, and the mean values (and standard errors) are of 70-90 observations each. The fluctuations were large, especially under the intermittent leaching regime, mainly due to the variations in the volumes of the leachate (see Fine et al., 2002a for details). The concentrations of nitrogen (mean and standard errors) in the irrigation water and in the leachates from the lysimeters are presented in Figure 2, either for the entire 257-day irrigation period (LF 1 and LF 0.2) or for the last 126-days period (Aug. 1 to Dec. 5; intermittent leaching).

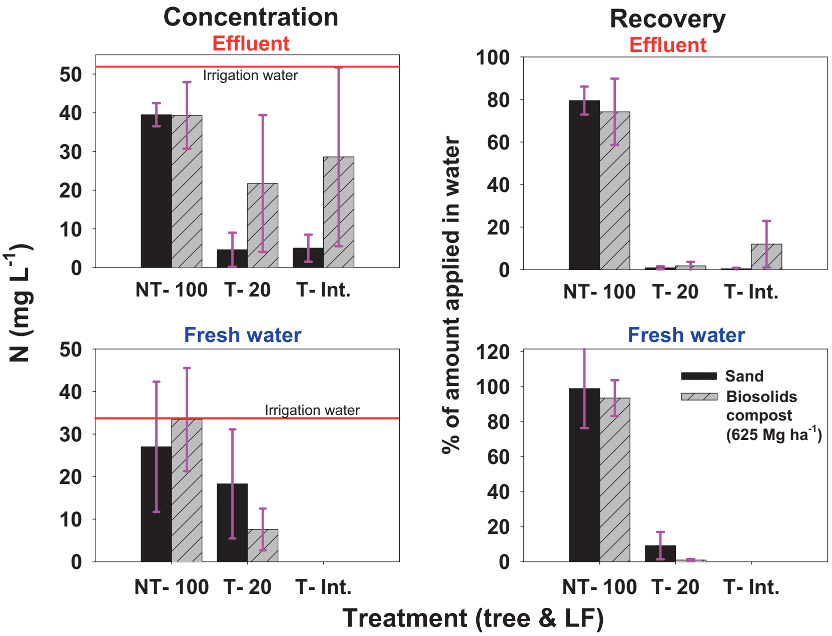

Figure 2. Nitrogen concentrations and recoveries in leachate from sand-packed lysimeters as affected by type of irrigation water, biosolids compost addition (at rate equivalent to 625 Mg/ha in the upper 20-cm layer), presence of a *Eucalyptus camaldulensis* tree and leaching regime (NT: without a tree & LF 100%; T: with a tree & LF 20% or intermittent leaching).

The data is from the 2nd year of the study. Irrigation was 3 times daily, every day. Number of lysimeters per treatment was 2 (not planted) or 3-10 (planted). Leachate was collected in 1-3 weeks intervals between March and December (intermittent leaching started in August). Individual recoveries were calculated separately for each lysimeter and leaching event. Values are mean and standard errors.

Nitrogen recoveries in the leachate from the freshwater, fertigated lysimeters without a tree were ca. 100% of the amounts applied (Figure 2A), and the corresponding value in the effluent amended not-planted lysimeter was close to 80%. Noteworthy is that the BC made no difference at this stage, being neither source nor sink for nitrogen. The difference in recoveries between effluent and fresh water irrigation resulted from physical filtration (77% of the OC in the effluent was >0.45 μm; Fine et al., 2002a) and biological immobilization and denitrification. It is quite likely that under effluent irrigation at LF 1, partial reducing conditions could occur in some parts of the soil profile. Hence, denitrification could have been the primary loss mechanism (Feigin et al., 1991; Master et al., 2003). However, a 15% mineral N loss is not unique to effluent irrigation. Denitrification losses of ^{15}N-labeled KNO_3 and clover residues that were incubated in a field-moist soil were 16% and 10% of the N applied, respectively (Muriuki et al., 2001). This is in the same range as we report here and as was reported by Feigin et al. (1991) and Master et al. (2003).

The recovery of N in the leachates from planted lysimeters was virtually zero, under all combinations of soil amendment, water types and leaching regime. Similar results were obtained by Polglase et al. (1995) for an effluent irrigated pine plantation. This efficient capture of N was in spite its constant supply and the shallow soil profile. Most all the N was probably intercepted by the trees, yet some surely denitrified. The rate of N supply seems not to have limited tree growth. We derived this from the fact that reducing the water application rate (at intermittent leaching) reduced the N supply but did not necessarily decrease the sizes of the trees (Table 2).

3.3 Leaching of Applied Phosphorus

The mean seasonal concentrations of phosphorus in the irrigation water and in the leachates from the lysimeters during the entire irrigation period are presented in Figure 3 for the same 10 treatments as above. Under fresh water fertigation, P concentrations were considerably lower in the leachate than in the irrigation water. They were 5.5 and 5.9 and 8.1 and 8.6 mg/L in at LF 1 and 0.2, in the sand and in the sand + BC treatments, all respectively. The concentrations seemed to be determined more by soil solution chemistry (namely, by precipitation) than by plant uptake. P concentrations in the leachates from the effluent irrigated lysimeters were higher, and at the lower leaching regimes (LF 0.2 and intermittent leaching) they were same or higher than those in the irrigation water. In most all the combinations, the addition of the BC somewhat increased the concentrations of P in the leachate, but the extent of increase did not reflect the amounts of P that were added.

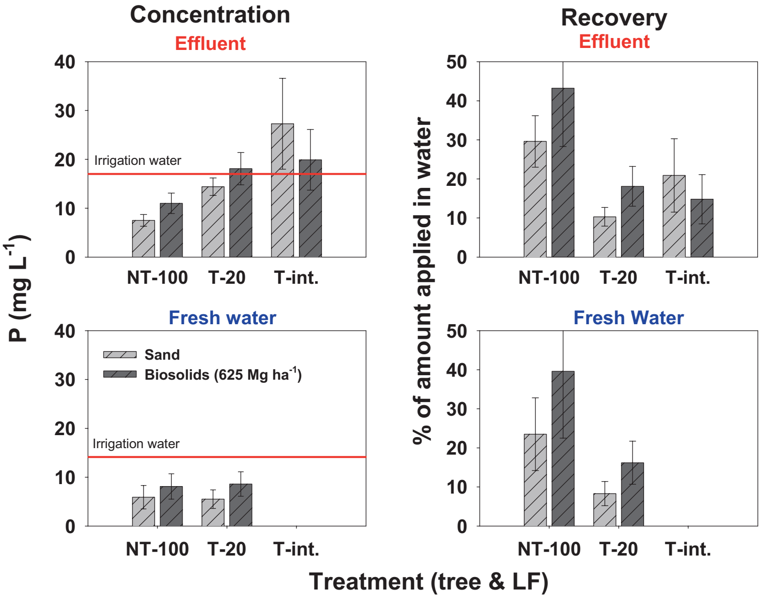

Figure 3. Phosphorus concentrations and recoveries in leachate from sand-packed lysimeters as affected by type of irrigation water, biosolids compost addition (at rate equivalent to 625 Mg/ha in the upper 20-cm layer), presence of a *Eucalyptus camaldulensis* tree and leaching regime. (NT: without a tree & LF 100%; T: with a tree & LF 20% or intermittent leaching). Other details are as in Figure 2.

Phosphorus recoveries in the leachate (as % of the amounts applied in the irrigation water) under both fresh water and effluent irrigation were 23-43% and 8-21%, without a tree in the lysimeter with a tree, respectively (Figure 3). The tree did take up P and decreased its leaching, however further reduction of the leaching (from continual 20% to intermittent) did not decrease the amounts leached. Instead, it only increased the concentrations in the leachate. This too demonstrates the importance of soil solution chemistry in controlling P solubility. The lack of soil desiccation under the irrigation/leaching regime used did not favor P precipitation in the soil. Furthermore, the main counter ions in the system that could help precipitate P were calcium and magnesium. Despite that their concentrations in the system were rather high (e.g., mean concentrations of Ca under effluent irrigation at LF 0.2 and intermittent leaching were 13 and 23 mmol$_c$/L, respectively), the high overall salinity (below, Figure 4), and the high concentrations of bicarbonate (not shown; originating mostly from roots and microbial respiration) and of DOC (Fine et al., 2002a), all coincided to reduce Ca and Mg activities and allow migration of substantial amounts of ortho-phosphate in the soil profile, and its leaching.

The addition of the BC at loading rate equivalent to 625 Mg/ha (with 9.6 Mg P/ha) added 240 g P to the upper soil layer of the lysimeter. This amount of P was 2-3 times more than the overall amount of P that was added in the irrigation water during the irrigation season (80-120 g P/lysimeter). Still, the BC did not consistently increase P leaching from the lysimeters (Figure 3) or it was not substantial, especially with a tree in the lysimeter. Inasmuch as this was the second year of the experiment, the small extent of leaching might represent low solubility and bioavailability of phosphorus in the BC. This is confirmed by the low P leaching observed by Elliott et al. (2002) for six BC in a column leaching study. The amounts leached were not statistically different from leaching from the unamended controls. It should be mentioned that leaching rate (1 pore volume in 4 months) and amounts of P applied (56-224 kg P/ha) practiced by Elliott et al. (2002) were substantially smaller than in the study herein.

Hence, we demonstrated that *Eucalyptus camaldulensis* in the system studied was very efficient in intercepting nitrogen and phosphorus applied by irrigation or in combination with BC. This was despite the excessive rates of nutrient application (in the water and biosolids) and soil leaching.

3.4 Effect of Leaching Regimes on Soil Salinity

Reduced leaching under the constant irrigation regime caused severe salination of the soil solution (Figure 4). Intermittent leaching was most detrimental, with mean seasonal chloride concentrations reaching ca. 0.1 M concentration, with salinity peaking at 215 mM. Spontaneous leaching occurred due to decrease in tree water uptake incidental with depressions in the potential evapotranspiration PET. Because irrigation continued, the soil salinity somewhat diluted. Under deficit irrigation, soil solution salinity could be increased even further (data not shown). Without a tree present in the lysimeter, the chloride concentration in the leachate (and soil solution) was as in th,e irrigation water and all the amount that was applied was fully recovered in the leachate under irrigation with the two water types. The trees did cause retardation of chloride migration, which accounted to some 30-40% of the amounts applied. In part, this retardation was due to reduced transport rate in the soil profile, as was evident from the smaller recovery (and greater salinization) under the intermittent leaching regime compared with leaching at LF 0.2. In part, the retardation was due to uptake of chloride by the trees. It should be mentioned that chloride was only one anion to contribute to soil solution salinity, sulfate and bicarbonate were other dominant anions that enhanced soil solution salinization even further (data not shown). Note also that the BC had no effect on the salinization (at least this stage).

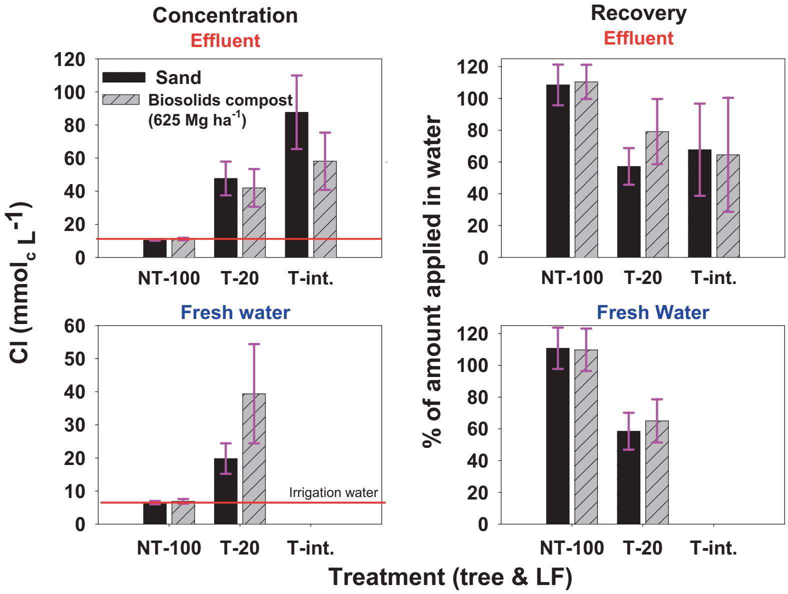

Figure 4. Chloride concentrations and recoveries in leachate from sand-packed lysimeters as affected by type of irrigation water, biosolids compost addition (at rate equivalent to 625 Mg/ha in the upper 20-cm layer), presence of a *Eucalyptus camaldulensis* tree and leaching regime (NT: without a tree & LF 100%; T: with a tree & LF 20% or intermittent leaching). Other details are as in Figure 2.

Hence, *Eucalyptus camaldulensis* is very resistant to increased salinity and it continues to be an efficient sink to both irrigation water and N at high soil salinities (in the range of 100 mM and more).

3.5 Leaching and Redistribution of Heavy and Trace Elements

The trends regarding the leaching of trace elements, their uptake by the trees and redistribution in the soil profile were common to most all the elements that were studied (i.e., Cu, Zn, Ni, Cd, Pb, Cr, Co, and Mn). These will be exemplified with Cu. The mean concentration of Cu in the leachate (values are for the entire 257-day period) without a tree in the lysimeter (LF 1) under fresh water irrigation was same as in the irrigation water, and it was somewhat higher with a tree (LF 0.2) (Figure 5). The BC (at 625 Mg/ha) increased the concentration, especially without a tree. Similar trends were observed under effluent irrigation; the concentrations were either lower or in the same range as in the fresh water irrigation (Figure 5).

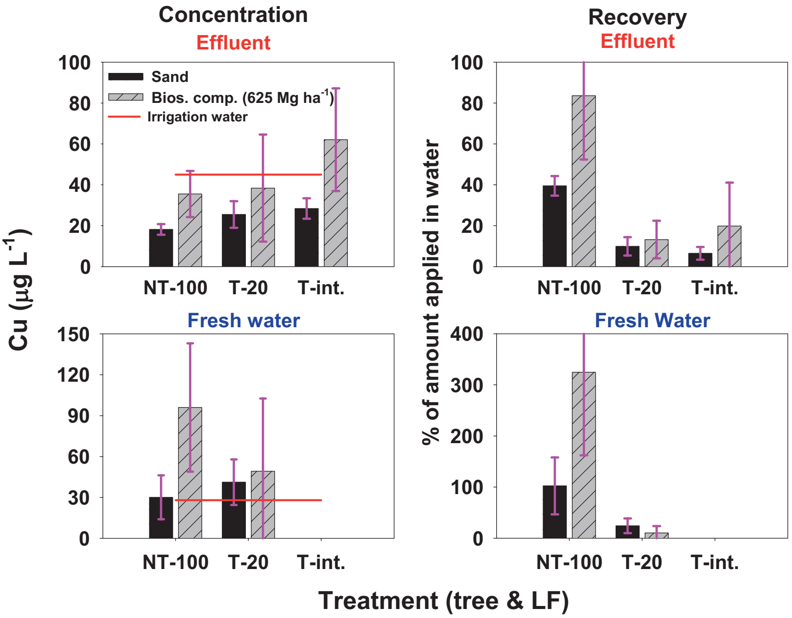

Figure 5. Copper concentrations and recoveries in leachate from sand-packed lysimeters as affected by type of irrigation water, biosolids compost addition (at rate equivalent to 625 Mg/ha in the upper 20-cm layer), presence of a *Eucalyptus camaldulensis* tree and leaching regime (NT: without a tree & LF 100%; T: with a tree & LF 20% or intermittent leaching). Other details are as in Figure 2.

Copper recovery in the leachate was calculated with respect to amounts of Cu applied in the irrigation water. The recovery without a tree in the lysimeter was 2-3 times larger with BC than without. Still, it was smaller under effluent irrigation (40-80% of the amount applied in the water has leached) than under fresh water irrigation (100-310%; Figure 5). With a tree present in the lysimeter, Cu recovery in the leachates was <25% and <15% of the amounts applied in the fresh water and effluent irrigation, respectively, and BC made no difference.

The mean amounts of Cu that were taken up in the tree tops under effluent and fresh water irrigation were similar, 40 mg Cu/tree. In the presence of the BC the uptake was 72 and 104 mg Cu/tree under effluent and fresh water irrigation, respectively. These accounted for 13 and 26%, and 30 and 72% of the amounts of Cu applied in the irrigation water, all respectively. The amounts of Cu were approximately 0.8-1.1% of the Cu load in the biosolids.

The results indicate that the tree-soil system efficiently intercepted effluent- and biosolids-applied Cu. The concentrations in the leachate from the effluent treatments were well below the standards for water for long-term irrigation (200 μg/L). The data also indicates that heavy metals in the BC were rather non-bioavailable, and that inasmuch as their bioavailability was increased by the EDTA (contained in the fresh water), the increase was small in absolute values (Figure 5). The effluent DOC was even less effective in rendering the BC Cu more bioavailable (Fine et al., 1997; 2002b). This is perhaps not surprising taking that the respective concentrations of EDTA and OC in the irrigation water were 4.3 mg/L and 192 mg Cu/tree. However, both degradation and concentration changed the concentrations (and the properties) once these constituents were within the soil profile (Fine et al., 2002a). The often larger extent of metal removal (in the trees and by leaching) and translocation (not shown) in the planted, fertigated lysimeters could have resulted from the high capacity of the concentrated EDTA to bind trace elements (Fine et al., 2002a; Figure 4). EDTA and EDTA-metal toxicities might also explain the high tree mortality rate (42%) in these treatments (Greman et al., 2003). It should be mentioned that metals redistributed in the soil profile, but it has yet to be determined if this was governed by dissolution and reprecipitation or by mere migration of BC particles (Yingming and Corey, 1993).

We conclude that the soil – tree system efficiently arrested the leaching of trace and heavy elements that originated from the BC and effluent in the effluent irrigated system.

3.6 Survival and Leaching of Indicator Bacteria

This is dealt with in detail elsewhere (Fine et al., 2005). Briefly, we studied the leaching of the indicator microorganisms, FC (and fecal streptococci; data not presented). We expected that at three times daily irrigations and short residence time in the 70-cm soil profile (LF 1; residence time 0.8 - 2 days), leaching of the enteric bacteria would be rather undisturbed (Gerba et al., 1975; Frankenberger, 1985; Gantzer et al., 2001). However, this was not the case as complete or near complete retardation/elimination (recoveries of 0-3%) of the FC in the leachates has occurred (Figure 6A). Also unexpected were the substantial FC counts that were observed in the leachate from the planted lysimeters (at water residence times that ranged from 7 to > 40 days). The overall recoveries of FC cells in the leachates showed a bell shaped pattern, with low counts at LF 1 and at intermittent leaching (the latter was the consequence of the minimum leachate volumes), while at LF 0.2 the recoveries were yet 5-45% of the number of cells that were introduced with the effluent water (Figure 6B).

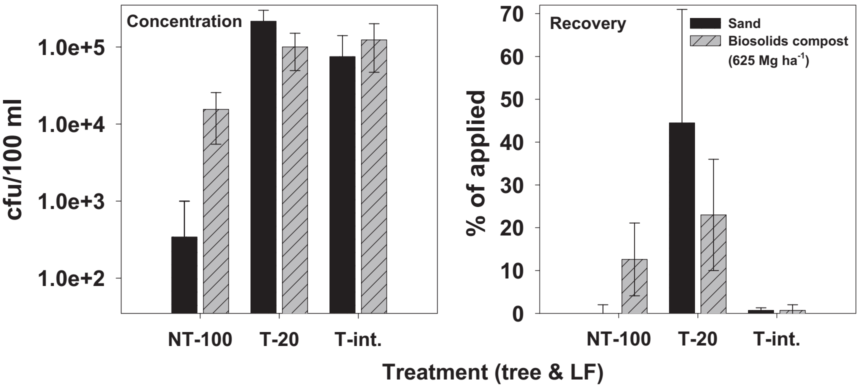

Figure 6. Fecal coliforms counts and recoveries in leachate from sand-packed lysimeters as affected by type of irrigation water, biosolids compost addition (at rate equivalent to 625 Mg/ha in the upper 20-cm layer), presence of a *Eucalyptus camaldulensis* tree and leaching regime (NT: without a tree & LF 100%; T: with a tree & LF 20% or intermittent leaching). Other details are as in Figure 2.

We hypothesize that this seeming discrepancy was affected by two main factors: (i) the concentrations of biodegradable OC in the leachate (and soil solution), and (ii) the relative rates of bacteria re-growth vs. predation upon. High BOD to support intense regrowth rates would support bacteria survival and leaching. Indeed, we found that without a tree in the lysimeter the BOD often completely depleted within the soil profile, probably ceasing to support re-growth. Yet, with a tree in the lysimeter, tree root exudates and slough-offs and effluent-derived TOC, both concentrated within the soil profile often to OC values higher than in the effluent itself (Fine et al., 2002a). Substantial BOD's were also present (data not shown). Hence, while without a tree predation seemed to dominate the fate of the FC's, with a tree, regrowth was to an extent that allowed for enough FC survival and leaching. This no new mechanism (Gerba et al., 1975; Gilbert et al., 1976; Ekelund and Rønn, 1994; Rønn et al., 2002), but it has yet to be demonstrated in the current study. It should be noted that while the soil solutions properties under LF 1 would have favored FC leaching (by high flow rates and relatively low salinity), under reduced leaching regimes (low flow rates, high ionic strength) retardation was expected to be mote dominant.

Hence, we conclude that under severely reduced leaching regime (intermittent leaching), the soil – tree system efficiently limits the leaching of indicator enteric bacteria. We also conclude that bacteria capable of regrowth are not necessarily good indicators of the behavior of pathogens in the soil environment.

4. SUMMARY AND CONCLUSIONS

The present simulation study performed under conditions extremely conducive to leaching, including exceptionally high water heads (equivalent to 21-31 m/yr) and a quartzitic dune sand for soil. Hence, under LF 1 and LF 0.2, the entire soil profile was continually purged. Under the intermittent leaching regime, soil purging decreased with increasing soil depth, but there was not much difference in the extent of flushing of the upper soil layers between the different leaching regimes. Furthermore, the BC was applied at 3.3 and 16.6 times the maximum permitted annual rates and the sewage effluent was loaded with nutrients, organic matter and microbes. Still, the soil - *Eucalyptus* system was very efficient in intercepting the environmental contaminants. This was demonstrated with respect to the virtually complete interception of nitrogen and near complete interception of phosphorus and heavy metals within the soil-plant system. Inasmuch plant uptake data was not presented here (except for Cu), it seems that the constituents of the BC were not bioavailable and they also did not appear in the leachate. The resistant of biosolids-borne phosphorus and heavy metals to weathering is and issue of ensuing studies (e.g., Elliot et al., 2002).

The system effectively filtrated out the indicator fecal bacteria (FC and FS; the data on the latter were not presented). We interpreted the data as demonstrating a bio-filter, through predation control of migration of the exogenous bacteria in the soil profile. Under certain circumstances, at frequent daily irrigation at LF 0.2, the microbes leached rather readily, perhaps via regrowth-dependent migration. The microbes were eliminated (or nearly so) under intermittent leaching and at its LF 1 counterpart. These results suggest that under real plantation conditions, with either intermittent leaching or rather deficit irrigation, fecal microorganisms will not migrate below the *Eucalyptus* root zone.

However, the combination of high water head and retarded leaching regimes can promote undesired changes of soil properties, and adversely affect soil and plant performance, thus limiting the long-term utilization and sustainability of the system. We demonstrated this elsewhere with respect to concentration of recalcitrant effluent-derived OC in the soil solution (Fine et al., 2002a) and we also discussed it above with respect to salinity buildup in the soil solution. We should mention that soil sodicity tends to escalate by soil purging with effluent and by soil salinization (Shainberg et al., 1980;

Evangelou and Marsi, 2003). However, natural attenuation mechanisms were shown to revert soil sodicity in the field (Agassi et al., 2004).

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PHYTOREMEDIATION OF EXPLOSIVES IN TOXIC WASTES

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Abstract: Selected emergent plants (helophytes) *Phragmites australis, Juncus glaucus, Carex gracillis* and *Typha latifolia* were successfully used for degradation of TNT (2,4,6-trinitrotoluene) under *in vitro* conditions. The plants took up and transformed more than 90% of TNT from the medium within ten days of cultivation. The most efficient species was *Ph. australis* which took up 98% of TNT within ten days. The first stable degradation products 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-4,6-dinitrotoluene (2-ADNT) were identified and quantified. TNT degradation products and their compartmentalization in plant tissues were evaluated after two weeks of cultivation using [^{14}C] TNT. Forty one percent of ^{14}C was detected as insoluble or bound in cell structures: 34% in roots and 8% in the aerial parts. The results were verified in pilot constructed wetland for cleaning explosive containing waste-waters as a necessary step *prior* real scale-up application.

Key words: phytoremediation; TNT; biodegradation; emergent plant; autoradiography

1. PHYTOREMEDIATION

Phytoremediation, one of the new biotechnologies, uses plants and their associated rhizospheric micro-organisms to remove, degrade, metabolize or detoxify contaminants including pesticides, metals, radionuclides, explosives, located in the soil, sediments, groundwater, surface water, and even the atmosphere (Chappell, 1997), These plants can be herbs, shrubs or trees, and they may be able to accumulate organics and heavy metals high

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above the levels found in nature (Brown, 1995; Ma et al., 2000). Numerous mechanisms exist by which plants can remediate contaminated sites - phytoaccumulation, phytoextraction, phytostabilization, phytotransformation, phytovolatilization and rhizodegradation.

Phytoremediation technology has two major advantages: it is relatively inexpensive and associated with minimal environmental disturbance. For these reasons phytoremediation has a high public acceptability. Worldwide-established phytoremediation-based systems appear to adequately and efficiently remove pollutants from various matrices at a comparatively low cost.

The other, long-established possibility has been removal of contamination by excavation and subsequent incineration. This is applicable only in cases when the contamination is very high as the process is very costly. On the other hand, levels as low as 10 mg/kg would be considered high enough to require remediation in the U.S.A. Generally, it is difficult to decide which technology should be applied at a certain concentration of contaminant. In the case of phytoremediation of e.g. TNT contaminated soils, the upper limit of 1,000 mg/kg of soil has been proposed for efficient application (Gerth et al., 2003). For such a low concentration less invasive alternative methods for removing of contaminants from the environment were suggested (Snellinx et al., 2002; Gerth et al., 2003).

In order to achieve successful, optimized phytoremediation a more systematic approach concerning the selection of plants and optimization of remediation processes is urgently required. Drawbacks and limits of the phytoremediation technique may actually be seen in the comparatively long time required for the completion of the process, in the limitation to surface soils (root penetration depth), climatic factors modulating the viability of the plants and thus the remediation success, the toxicity of the pollutant and its degradation products to the plants and the possible need for soil amendment. Therefore the space opens for using techniques of molecular-biological and genetic to improve properties of (crop) plants and efficiently battle with the spread of contamination. Despite anti-GMO activities in the EC, genetically modified plants suited for phytoremediation represent new generation of GM plants. The genetically modified plants could be useful for efficient cleaning of wastewater as well.

1.1 Phytoremediation of Organic Xenobiotics

Phytoremediation of organic xenobiotics is generally based on mineralization or more frequently on degradation/transformation of the xenobiotics to environmentally less dangerous compounds, which are fixed in cell compartments or stored in the vacuole as soluble products or exuded back to the environment. Affection of the metabolic pathway by suppression or enhancement of expression of enzymes leading to degradation of xenobiotics by genetic modification might be a useful tool for efficient removing of xenobiotics from the environment.

1.2 Phytoremediation of Explosives

The contamination of the environment by explosives, especially by nitroesters and nitroaromatics (NACs), is a worldwide environmental problem since enormous amounts of these compounds were produced during World War I and II. Most contaminated sites are located at ammunition factories and other places where these compounds were handled. This involved open detonation and burning of explosives at army depots, evaluation facilities, artillery ranges, and ordnance disposal sites (Rodgers and Bunce, 2001).

Degradation pathway of nitroesters such as nitroglycerine (NG) and pentaerytritoltetranitrate (PETN) were already elucidated. By the analysis of products and stoichiometry of both processes, glycerol and pentaerythritol were identified as end products (Schwitzguebel and Vaněk, 2003). These compounds can eventually be used by plant cells as a carbon source (Maestri et al. 1991).

The degradation of TNT in plants is still not fully explored, although principal degradation processes, such as reduction leading to the formation of monoaminodinitrotoluenes (Palazzo and Leggett, 1986; Görge et al., 1994), oxidation (Bhadra et al., 1999a) and conjugation (Bhadra et al., 1999b) reactions have already been described. Monoaminodinitrotoluenes belong to the main degradation products detectable in the medium.

1.3 Phytoremediation of TNT

In the Czech Republic, as well as in Germany, the main contaminant, especially of former and present ammunition factories, is TNT. The most serious contamination was recognized as a result of production as well as of use of 2,4,6-trinitrotoluene (TNT), mainly because of vast amount of water necessary for manufacturing this compound made it necessary to locate the factories near natural sources of water – mostly rivers. The environment around large rivers is usually characterized by sand-rich soil that allows easy seepage of rainwater. NACs are therefore continually washed out from the contaminated soil and subsequently contaminate groundwater. As the sandy soil is not rich in humic substances, the capacity for binding of TNT and its derivatives is limited (Ahmad and Hughes, 2002; Wang et al., 2002). The persistency of NACs in the environment is due to resistance to biodegradation caused by their high toxicity for most living organisms

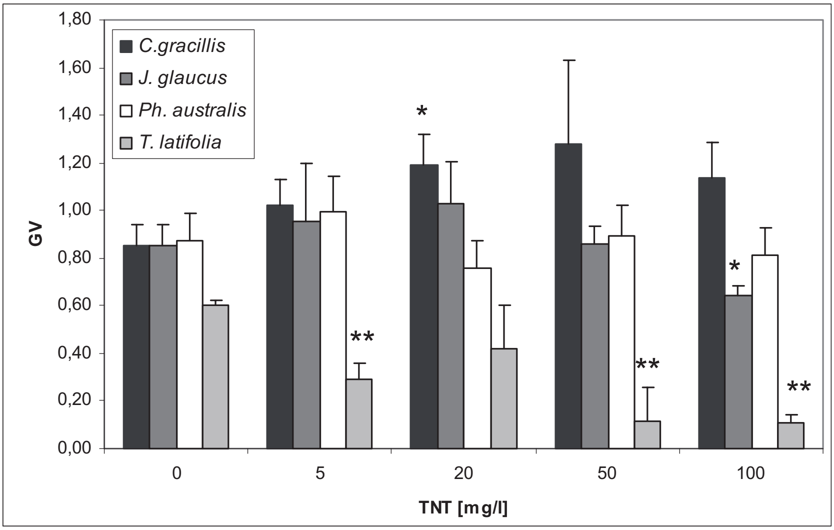

Figure 1. Phytotoxicity test of TNT. The inhibition/induction effect of TNT on plants is expressed by growth value (GV= (wt - w_0) / w_0; w_0 – inocula, wt – at time "t").

(Lachance et al., 1999), low solubility in water (Ro et al., 1996), and consequently the absence of natural attenuation.

The first accounts of the removal of TNT from groundwater by emergent and submersed plants were published by Best et al. (1999). In our laboratory we focused our effort on the phytoremediation of water contaminated by TNT comparing the effectiveness of TNT degradation by selected helophytes (*Phragmites australis, Juncus glaucus, Carex gracillis and Typha latifolia).* A model *in vitro* system was used for studying degradation processes in plants without the influence of microorganisms.

The effect of TNT on the individual species was determined by comparing growth value at four different concentrations (Figure 1). TNT induced the growth of *C. gracillis* and *J. glaucus* up to concentrations of 50 mg/L and 20 mg/L, respectively. These two species exhibited the highest resistance to TNT whereas the growth of *Ph. australis* did not show significant differences in relation to TNT concentration. The growth of *T. latifolia* was inhibited (IC50 301 mg/L). The IC50 concentration for the remaining species could not be determined due to the low solubility of TNT in water. The disappearance of TNT from the medium was connected with the uptake of TNT into the plant tissues and its transformation into degradation products. About 80% of TNT was taken up from the medium within 3 d and at least 90% of TNT was taken up by the plant species *Ph. australis*, *T. latifolia* and J. *glaucus* within 10 days (Figure 2).

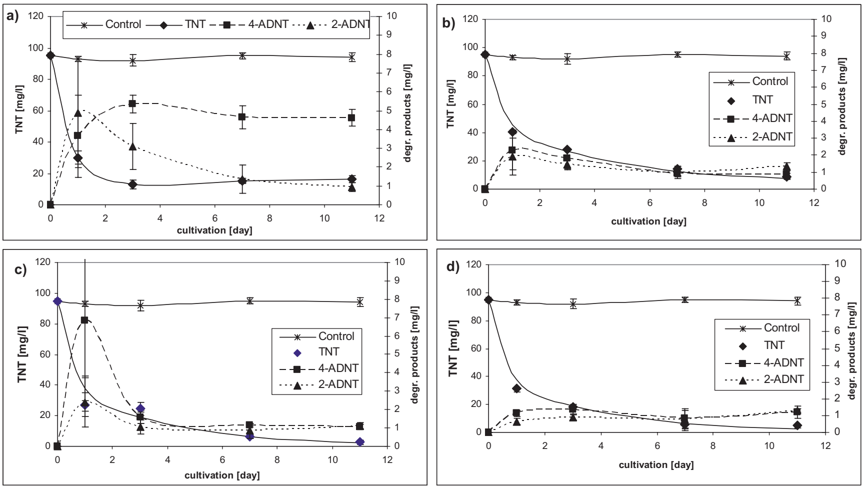

Figure 2. Degradation of TNT by in vitro cultivated water plants *C. gracillis* (a), *J. glaucus* (b), *Ph. australis* (c), *T. latipholia* (d).

In these experiments, aminodinitrotoluenes were found to be the main degradation products of TNT. Known products of reductive degradation, such as 4-hydroxylamino-2,6-dinitrotoluene, diaminonitrotoluenes or azoxytetranitrotoluenes (Sun et al., 2000) or the products of oxidative degradation, e.g. trinitrobenzene (TNB) (Nepovim et al., 2004) have not been detected in our experiments.

Only small differences in the concentration of ADNTs in the media treated by the individual plant species were observed in the course of cultivation, with the exception of the first day, when the reed released the highest amount of 4-ADNT. In all the media tested, concentrations of 4-ADNT exceeded those of its isomer. This was in accordance with results published earlier (Nepovim et al., 2004) and was recently theoretically supported by results of quantum chemistry calculations at the SCF level (HF/6-31G pp) (Huang and Leszczynski, 2002). The latter study showed that the preference of reduction at the 4-position arises from the preferred protonation at this site (a 3.7 kcal/mol difference) as compared with the 2-position, even though the subsequent intermediates leading to 2-ADNT are slightly more stable than their 4-position counterparts (Huang and Leszczynski, 2002).

A number of unique degradation products resulting from oxidative processes, such as 2-amino-4,6-dinitrobenzoic acid, 2,4-dinitro-6-hydroxybenzyl alcohol, 2-N-acetoxyamino-4,6-dinitrobenzaldehyde and 2,4-dinitro-6-hydroxyltoluene, were identified by Bhadra et al. (1999a). However, it is not clear if the formation of these products is the result of

Figure 3. Distribution of radioactivity in reed. A photograph of a plant (a) and its autoradiogram (b). Plants were treated by [14C]TNT (1.02 MBq/L) for 14 days. A plant was washed, dried and exposed to film for 3 weeks.

plant or microbial activity and/or the synergistic effect of metabolism of both organisms. Only *in vitro* conditions can ensure that the microbial effect is excluded from those of plant metabolism, with the theoretical exception of possible endophytic bacteria. Many authors studied the fate of TNT and its metabolites in plants using [^{14}C]TNT (Hughes et al., 1997; Bhadra et al., 1999b; Sens et al., 1999; Sun et al., 2000) and found, that metabolism of TNT is generally concentrated in roots (Sens et al., 1998, 1999; Best et al., 1999).

Our results showed that about 63% of ^{14}C localized in the roots of *Ph. australis* (Figure 3 and 4) was bound and 37% was acetone-extractable (Figure 3b). The HPLC analysis of acetone extract of plant tissues showed that no TNT was determined, therefore all TNT was transformed. On the basis of this result we came to the conclusion that TNT was not adsorbed on the root surface (Nepovim, 2005).

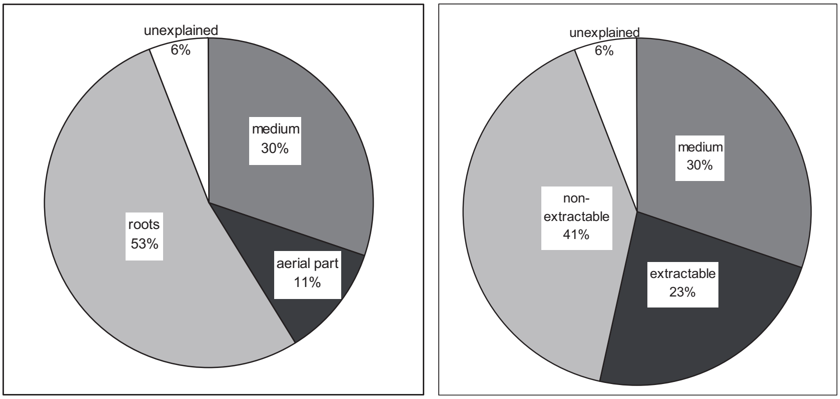

Figure 4. Distribution of radioactivity in particular parts of *Ph. australis* after cultivation in liquid medium (a) and its availability from plant tissues.

Sens et al. (1999), who studied the degradation of TNT in wheat (*Triticum aestivum*), found that more than half of uptaken ^{14}C (57%) was bound to the cell wall and the rest was detected in the cytoplasm as soluble compounds. The lignin fraction contained 27% of uptaken ^{14}C which was comparable to the results of a study on bushbean (*Phaseolus vulgaris*) in which 20% of uptaken ^{14}C was bound to lignin (Sens et al., 1998). The above-mentioned studies showed that the metabolism of TNT in plants cannot be generalized, especially when considering the formation of a wide spectrum of degradation products at different concentrations. It is possible to propose a major degradation pathway, but the formation of end products differs among plant species depending upon the available enzymes involved in detoxification processes.

1.4 Pilot Constructed Wetland

The helophytes were used for wastewater treatment in constructed wetlands by Brix (1994) or Langergraber and Haberl (2001). Based on the promising results of laboratory experiments we decided to use the selected emergent plants for constructed wetlands in the temperate zone of Central Europe and to evaluate the possibility of cleaning of TNT contaminated water. We constructed a model of wetland consisted of 4 plastic containers 1000 l volume each was set-up and tested it for cleaning of explosives containing waste-waters (Figure 5 and 6). This cleaning process was, after subsequent optimization, patented recently (Vanek, 2005).

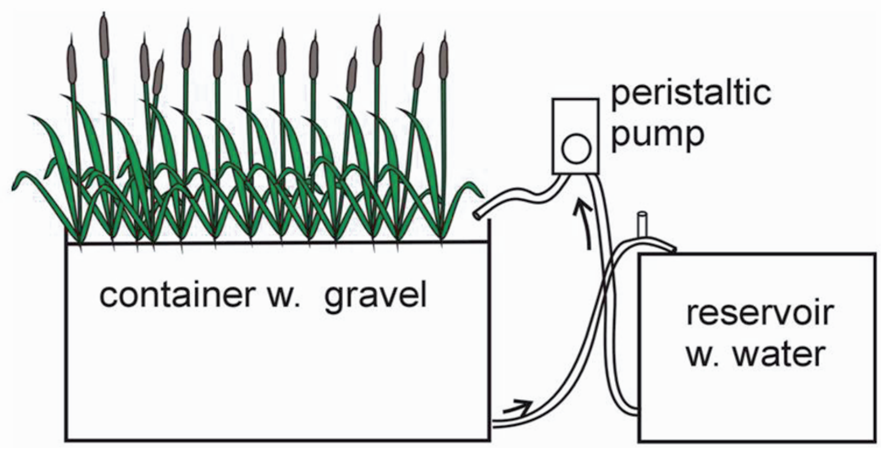

Figure 5. Scheme of pilot constructed wetland.

Figure 6. Pilot constructed wetland – each container volume is 1 m^3.

2. CONCLUSION

All tested emergent plants (helophytes) showed a potential for removal of TNT from contaminated water under *in vitro* conditions with small differences in the formation of the major degradation products – monoaminodinitrotoluenes. Most of TNT degradation products (using ^{14}C-radiolabelled TNT) were localized in the roots of reed (53% of total radioactivity) as insoluble compounds (33% of total radioactivity).

Based on the above-mentioned results, *Ph. australis* seems to be the best candidate for practical application. This idea is supported by the fact that it forms prolific biomass necessary for efficient biodegradation and is able to grow almost everywhere.

The fact, that most of TNT degradation products are localized in roots decreases the possibility of the contamination of the food chain, which represents another practical advantage of *Ph. australis*.

This possibility was verified during pilot-scale installation of constructed wetland for cleaning of explosives containing waste-waters in a producing factory area.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

ADNT: 4-amino-2,6-dinitrotoluene; DANT: two diaminonitrotoluene isomers; NACs: nitroaromatic compounds; TNT: 2,4,6-trininitrotoluene

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FLOATING AQUATIC MACROPHYTES AS A DECONTAMINATION TOOL FOR ANTIMICROBIAL DRUGS

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Abstract: Plant capability to lower antimicrobial drug concentration, such as sulfonamide (Sulfadimethoxine) and a quinolone (Flumequine), widely used in intensive farming, has been tested in laboratory models already set up in previous experiments. The aim of this work was to determine phytoremediation capability of different macrophytes, *Azolla*, *Lemna* and *Pistia*, by evaluating the effects on both plant growth and drug uptake/degradation. The three plant models survived even at very high drug concentrations although toxic effects were evident, particularly in the case of *Pistia* that died after 3 weeks at the highest concentrations. Furthermore, the three plant models are able to absorb and store drugs, particularly *Azolla*, which showed high drug uptake and degradation. These results lead us to hypothesise an important role for these plants as a bioremediation technique in the future.

Key words: antibiotic, *Azolla*; flumequine; *Lemna*; phytoremediation; *Pistia*; sulfadimethoxine

1. INTRODUCTION

Antimicrobial drugs are widely used in intensive farming (including aquaculture) both as feed additives and in mass therapy. They are administered as medicated feed, slowly absorbed and eliminated with faeces, ending up into the environment. After elimination from the body, these drugs (and their metabolites) can maintain significant residual activity and toxicity

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in manure for long times. As a consequence of waste disposal, drugs may contaminate natural ecosystems (Halling-Sorensen et al., 1998; Jørgensen and Halling-Sørensen, 2000).

The widespread and massive utilisation of antibacterial drugs in intensive farming poses the question of their environmental fate and side effects. The release of drugs into the environment is responsible for the contact of wild organisms with amounts of single (or a few) active pharmacological materials. While human wastes usually contain low amount of many different drugs, animal waste contains high amounts of usually one or few antibacterial drugs.

Macrophyte-based treatment appears to be highly competitive among the existing refined treatment methods; the type of aquatic plant used can make a significant difference in the pollutant removal (Reddy, 1983; Gersberg et al., 1986). The water velvet is an aquatic fern known to absorb pollutants (Gaur and Noraho, 1995; Forni et al., 2001) and to be quite resistant to antibiotics (Forni et al., 1991; Forni et al., 2002). Duckweed is another free-floating aquatic plant known to absorb pollutants, and under study in our lab to evaluate its resistance to Flumequine (Cascone et al., 2004). The floating macrophyte water lettuce, is another promising bioremediation candidate, known to absorb heavy metals (Maine et al., 2001). Therefore, the activities of plants may offer a viable mean for *in situ* remediation of drug contaminated wastewater as to lower drugs content even in tanks during manure maturation period. To lower drugs content a bioremediation model has been set up. This model involves floating macrophytes such as water velvet, duckweed and water lettuce (*Azolla filiculoides* Lam., *Lemna minor* L. and *Pistia stratiotes* L.) challenged against two antimicrobial drugs, Sulfadimethoxine (S) and Flumequine (F).

2. MATERIALS AND METHODS

2.1 Plants and Culture Conditions

Plants were cultivated outdoor at five S concentrations (0, 50, 150, 300, and 450 mg/L) and five F concentrations (0, 50, 100, 500, and 1000 µg/L) (Sigma, Milan) in 1/10 strength Hoagland mineral medium, N-free (*Azolla*) and added with 200 mg/L KNO_3 (*Lemna* and *Pistia*). Two different sets of experiments under the same experimental conditions were performed with and without plants. Plants were treated with Flumequine or Sulfadimethoxine. 5 g *Azolla* and *Lemna* and 20 g *Pistia* were inoculated in plastic bowls (30 cm in diameter) containing 1.5 L of the above mentioned medium added or not with drug. Care was taken to select *Pistia* plants of uniform size (about 1 g). All test concentrations were nominal (e.g., not measured analytically); a randomised block design was applied to the experiments. Culture media dilution was avoided by covering all the bowls with plastic sheets. Culture medium volumes were measured weekly and refilled with fresh Hoagland medium at the same drug concentration, up to starting level, to compensate the losses due to evapo-transpiration. Plants, were collected weekly, wiped off with paper tissue, weighed (fresh weight) and reinoculated into the bowls. Each treatment was performed in triplicate. Flumequine experiments lasted 5 weeks, while Sulphadimetoxine lasted 4 weeks, due to the different drug concentrations and consequent toxicity. Results are the average of three replicas.

2.2 Chemical Analyses

The concentrations of Flumequine and Sulphadimethoxine were quantified in the media and in the plants. Plants were weighed (fresh weight) at the end of the experiment, and dried at 50°C for 48 h to obtain dry weight. Liquid medium was directly processed.

2.2.1 Analysis of Flumequine (F)

Extraction procedure of Flumequine. F was extracted according to the method of Migliore et al. (2000).

Plant. 0.5 g of dry weight was homogenized by Ultraturrax in 20 ml ethyl acetate/n-hexane 3:1. The samples were centrifuged (3000 *g*, 15 min) and the supernatants were collected and cleaned up on 3 ml SPE-NH_2 columns (amino solid phase extraction, J.T. Baker, Netherlands) previously conditioned with 10 ml methanol + 6 ml ethyl acetate/n-hexane 3:1, after washing columns with 5 ml ethyl acetate/n-hexane 3:1, F was eluted by 12 ml acetonitrile/oxalic acid 0.025 M (pH 2) 8:2. This fraction was collected and dried under a stream of nitrogen.

Culture medium. 5 ml samples were acidified with 600 μl of 85% phosphoric acid and extracted with 3 + 2 ml chloroform. The organic phases were collected and dried under a stream of nitrogen. Residues were resuspended in 100 μl methanol and 50 μl was injected in the HPLC-Fluorimeter system.

Chromatographic analysis. A WatersTM 600 pump equipped with a Merck column Purospher^R RP-18 endcapped (5 μm) (4.6 × 250 mm) and a Fluorimetric detector (Waters 470 photodiode array detector 996) were used. The mobile phase was 0.035 M oxalic acid (pH 2.2)/acetonitrile (80%). The running condition was as follows: from 0 to 1 min isocratic run at 20%

acetonitrile, followed by linear gradient up to 80% acetonitrile in 14 min; then return to initial conditions in 3 min. The flow was 0.75 ml/min. A 50 μl loop was used. The detector was set at λ excitation 327 nm and λ emission 369 nm. Analyses were performed at room temperature. Reagents were HPLC grade (Merck).

Drug quantification. Recovery rates of the extraction procedure were calculated by spiking the control plants and culture media with known amount of Flumequine standard (62.5 ng) and applying this procedure 3 times to each control. Rates were higher than 90% for the culture media samples and 80% for the plants samples. Flumequine content was quantified by the external standard plot method (calibration curve between the concentrations of 7.5 and 125 ng injected; r = 0.998), lower limit of determination 20 ng/g dry weight (ppm).

2.2.2 Analysis of Sulfadimethoxine (S)

Extraction procedure of Sulfadimethoxine. S was extracted according to the method of Migliore et al. (1995).

Plant. 0.5 g dry weight were homogenized by Ultraturrax in 10 ml methylene chloride/acetone 1:1, added with sodium sulphate and sodium chloride, vortexed for 3 min and sonicated for 10 min. Samples were centrifuged (500 *g,* 5 min) and supernatants were collected, brought to 9.5 ml with methylene chloride/acetone 1:1 and 500 μl glacial acetic acid was added. The supernatants were cleaned up on 3 ml SPE-SCX columns (sulphonic acid solid phase extraction, J. T. Baker, Netherlands), previously conditioned with 10 ml methylene chloride/acetone/glacial acetic acid 47.5: 47.5:5. After washing columns with 3 ml water and 5 ml methanol, S was eluted by 6 ml of 2.5% NH_3 in methanol. This fraction was collected and dried under a nitrogen stream and the residue was resuspended in 100 μl methanol.

Culture medium. 5 ml of medium was adjusted to pH 5.5 with 1N HCl or 1N NaOH and extracted with 5 ml methylene chloride. The organic phase was dried under a nitrogen stream and the residue was resuspended again in 100 μl methanol.

Chromatographic analysis. The analysis were performed by HPLC. 50 μl of each sample were injected into the HPLC with a Symmetry column C18 (4.4 × 250 mm) and a UV/DAD detector (diode array detector) and an ion trap mass spectrometer were used under the following conditions. Mobile phase acetonitrile/H_3PO_4 0.017 M with diethylamine 0.5 g/L Run: from 0 to 1 min followed by linear gradient to 80% acetonitrile in 10 min; from 11 to 14 min isocratic run at 80% acetonitrile, then return to initial conditions in 2 min. Flow: 1 ml/min. Loop: 50 μl; detector settled at λ excitation 275 nm and λ. emission from 220 to 350 nm. Analyses were performed at room temperature. Reagents were HPLC grade (Sigma).

Drug quantification. Recovery rates of the extraction procedure were calculated by spiking the control plants and culture media with known amounts of Sulfadimethoxine standard and applying this procedure 3 times to each control. Rates were higher than 80% for the culture media samples and 60% for the plants samples. Flumequine content was quantified by the external standard plot method (calibration curve at concentrations of 10, 50, 100, 500, and 1000 ng; r = 0.998). The lower limit of determination was 10 ng/g dry weight (ppm).

3. RESULTS

3.1 *Azolla filiculoides*

S and F affected growth rate of the plants (Figures 1, 2); and drugs bioaccumulation was found (Table 1). The amount of drug residues remaining in the growth media and percentage removal were determined.

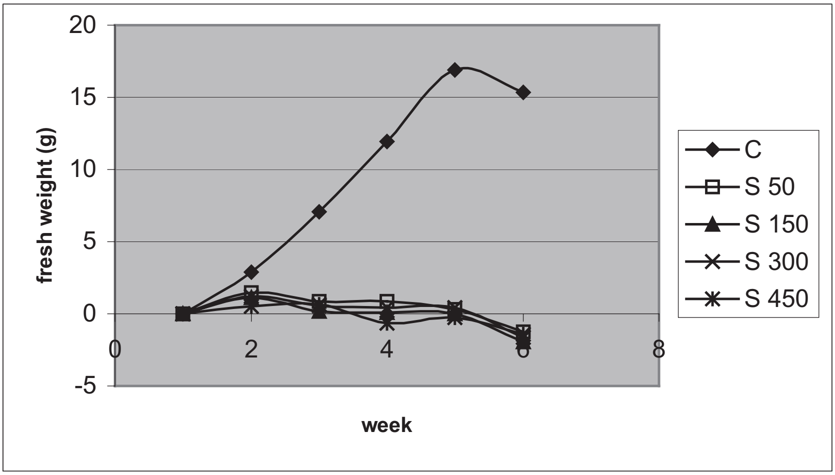

Figure 1. Growth of *Azolla* in the presence of S.

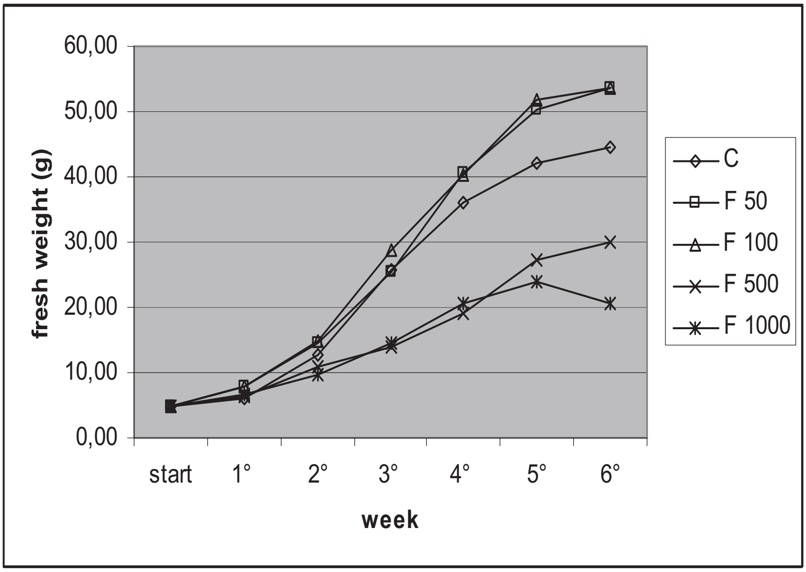

Figure 2. Growth of *Azolla* in the presence of F.

The comparison between tests with and without plants, under the same environmental conditions, showed that, in S treated batches, a higher proportion of drug was removed if the fern was present (from 56% at S 50 to 88% at S 450). Also in F-treated batches a higher proportion of drug was degraded if the fern was present (from 98% at F 50 to 96% at F 1000).

Table 1. Amount of S and F detected in the plants (μg/g dry weight)

Test S	*Azolla*	*Lemna*	*Pistia*	Test F	*Azolla*	*Lemna*	*Pistia*
50	**58.35**	2.21	**11.49**	50	**0.031**	5.20	**0.169**
150	**160.86**	4.11	**43.08**	100	**0.055**	14.50	**0.321**
300	**992.45**	7.24	-	500	**0.179**	53.40	**0.414**
450	**2012.09**	12.86	-	1000	**0.573**	94.50	**0.648**

3.2 *Lemna minor*

S and F affected growth rate of the plants (Figures 3, 4); the species was more tolerant to F. Drug bioaccumulation was found (Table 1). The amount of drug residues remaining in the growth media and percent removal were determined. The comparison between tests with and without plants, under the same environmental conditions, showed that, in S treated batches, a higher proportion of drug was removed if the duckweed was present (from 65% at S 50 to 73% at S 450). Similarly, also in F treated batches a higher proportion of drug was degraded in the presence of the duckweed (about 96% at all concentrations).

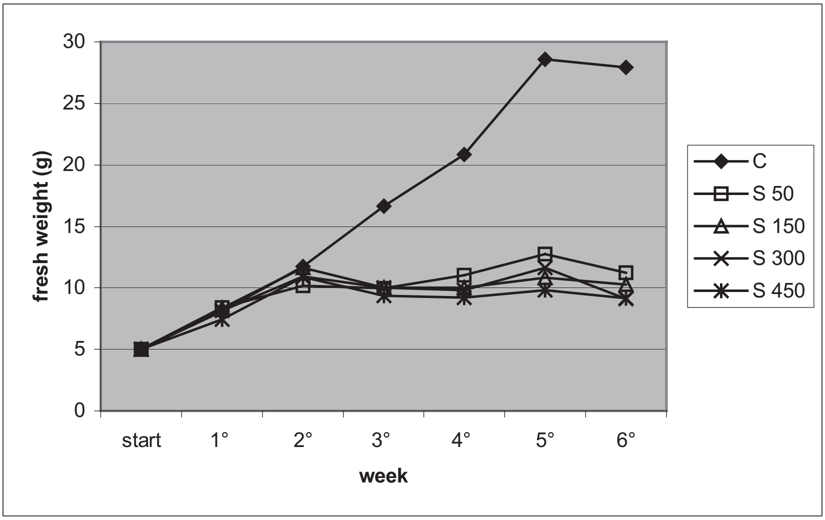

Figure 3. Growth of *Lemna* in S added medium.

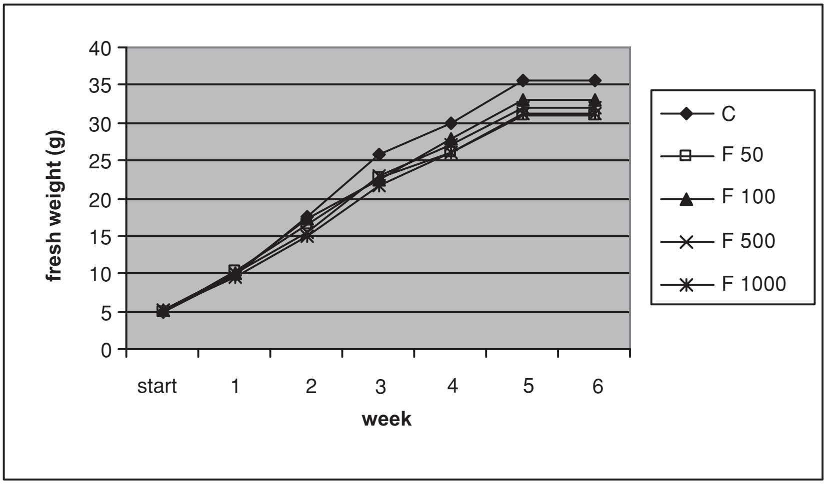

Figure 4. Growth of *Lemna* in F added medium.

3.3 *Pistia stratiotes*

S and F affected growth rate of the water lettuce (Figures 5, 6). The damage due to S is dramatic (plants died after three weeks at S 300 and S 450); the species was more tolerant to F. Drug bioaccumulation was found (Table 1).

The amount of drug residues remaining in the growth media and percentage removal were determined. The comparison between tests with and without plants, under the same environmental conditions, showed that in S treated batches a higher proportion of drug was removed if the *Pistia* was present (56.8% at S 50 and 66% at S 150). Also in F-treated batches a higher proportion of drug was degraded if the water lettuce was present (about 93% at all concentrations).

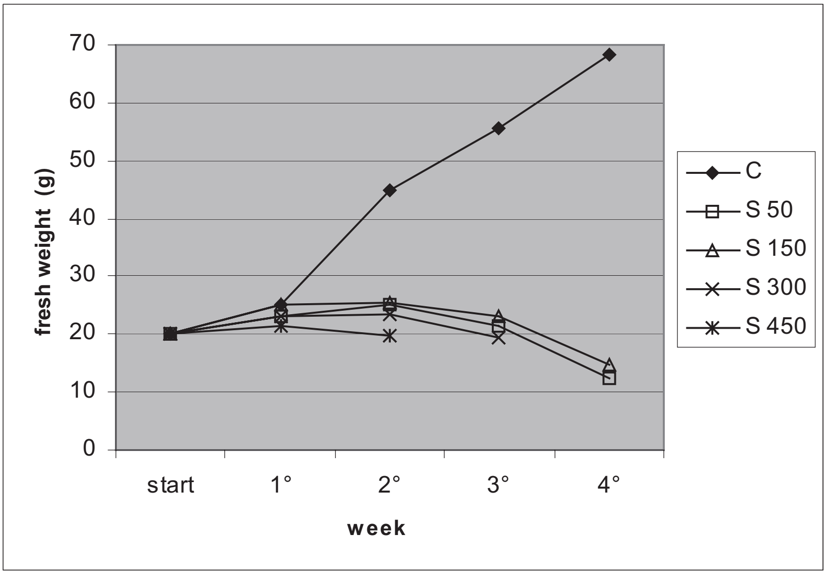

Figure 5. Growth of *Pistia* in S added medium.

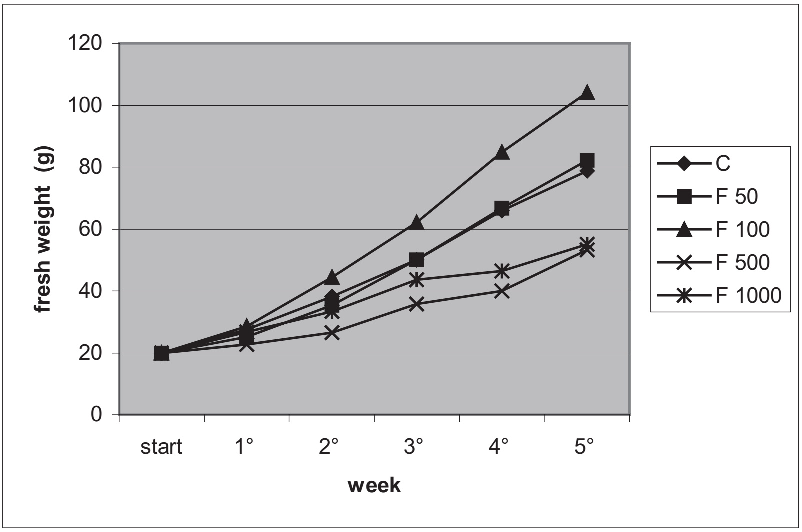

Figure 6. Growth of *Pistia* in F added medium.

4. DISCUSSION

Antimicrobial drugs, Sulfadimethoxine and Flumequine, affected growth rate of the three floating macrophytes tested as bioremediation tools; nevertheless plants are able to survive, with the exception of *Pistia* treated with S, that died at the highest concentrations after three weeks. This growth reduction depends on drug accumulation in the whole body of the three plants species; the order of decreasing accumulation capability is *Azolla* > *Lemna* > *Pistia* for both drugs.

The amount of drug remaining in the growth media with and without plants was also determined to define bioremediation efficiency of species; all plants are able to remove higher percentage of both drugs if compared to abiotic/microbial degradation, naturally occurring under the experimental system conditions. In any case, the final result in the experimental system (plant uptake/degradation plus abiotic/microbial degradation) lead up to 98 % of the drugs removed from the model system.

Because of plant resistance and drug removal capability, *Azolla* is the most efficient tool for Sulfonamides environmental monitoring and cleaning to decontaminate tank systems. It would be useful as a bioremediation technique in the lagoons for animal farming wastewaters storage, even in the case of dramatic variations of the antimicrobial load in the waste, as currently happens with alternation of treatment/no treatment periods. *Lemna* is the second candidate being highly efficient in Quinolone environmental monitoring and decontamination. *Pistia* is more sensitive than the other two macrophytes tested with the antimicrobial drugs, therefore its utilisation for Quinolone bioremediation may be suggested, while it is less useful for Sulfonamide.

5. CONCLUSIONS

In conclusion, *Azolla* and *Lemna* survival rates and trapping/degrading capacity (at concentrations of one order of magnitude higher than those expected in manure) constitutes a starting point to set up a suitable and easy tool for drug decontamination of wastewaters; the combined use of floating plants, including *Pistia*, may assure a complete bioremediation on the entire system. Furthermore, the multispecific approach, because of different sensitivity and seasonal growth rates could improve the existing refining treatment methods.

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PLANT TOLERANCE TO HEAVY METALS, A RISK FOR FOOD TOXICITY OR A MEANS FOR FOOD FORTIFICATION WITH ESSENTIAL METALS: THE *ALLIUM SCHOENOPRASUM* MODEL

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Abstract: There is a duality in plant tolerance and response to heavy metal stress. On one hand tolerant plants having heavy metal hyper accumulation potential could be beneficial in phytoremediation for clean-up of soil and water; on the other hand tolerant food crops, if exposed to heavy metals in their growth medium, may be dangerous as carriers of toxic metals in the food chain leading to food toxicity. Tolerant food crops could also be used for enrichment of the diet with essential metals. Heavy metal stress causes oxidative damage, but some reactive oxygen species (ROS) can participate in signal transduction pathways. A new hypothetical mechanism for the interaction of cupric ions and ascorbic acid with proteins in response to metal stress is presented. The dualities mentioned above are discussed. *Allium schoenoprasum* (Chives) is the model plant to be presented. Like other members of the *Allium* genus it has a wide spectrum of sulfur-containing compounds in high concentrations, this may render it more tolerant to heavy metals. Some of these compounds may function as reductants and/or chelators to prevent the toxic effect of the metal. The results presented, point to chives phytoremediation potential, but also to the potential risk in accumulation of heavy metals in a commonly edible plant.

Key words: phytoremediation; tolerance; cadmium; iron fortification; ascorbic acid-copper

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1. INTRODUCTION

This paper focuses on dualities which stem from plant tolerance to heavy metals (Figure 1). On one hand tolerant plants having heavy metals hyperaccumulation potential could be beneficial in phytoremediation for clean-up of soil and water. On the other hand tolerant food crops, if exposed to heavy metals in their growth medium, may be dangerous as carriers of toxic metals in the food chain leading to food toxicity.

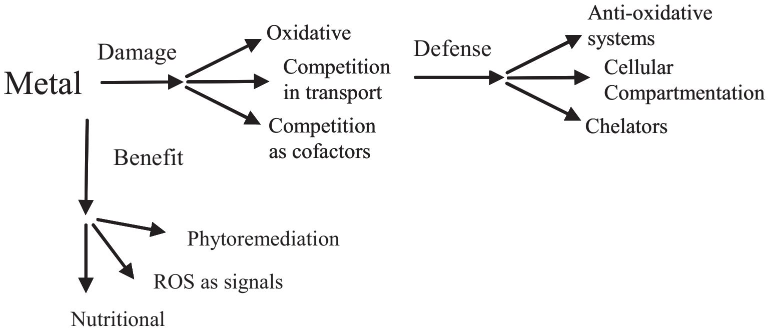

Figure 1. Schematic view of plant response to metal stress and some biotechnological aspects.

Still another aspect of this duality lies in the potential use of tolerant crops for fortification of food with essential metals (Guerinot and Salt, 2001). There is also duality in plant tolerance to heavy metals at the biochemical level, on one hand metals cause oxidative damage, on the other hand reactive oxygen species (ROS) can enter signal transduction pathways (Davletova et al., 2005). In this paper I shall discuss these dualities using *Allium schoenoprasum* (Chives) as the model system. Phytoremediation, iron fortification and a new hypothetical mechanism for the interaction of Cu and ascorbic acid with proteins in response to metal stress is presented.

2. METHODS AND MATERIALS

2.1 The *Allium Schoenoprasum* Model

Allium schoenoprasum (Chives) plants were grown hydroponically in 'aerated half strength Hoagland medium, with metals added as indicated in the tables (for experimental details see Barazani et al., 2004). The plants were grown in bunches of 25 seedlings each and were reharvested every 2 weeks by cutting the shoot approximately 3 cm above the bulb. This approach enabled uptake of metals from the medium over extended periods on the same root system (Figure 2).

Figure 2. Experimental system - hydroponically grown Chives.

3. RESULTS

3.1 Metal Accumulation in Chives

Treatment of chives with Ni, Co and Cd at 0.05 and 0.25 mM, showed that the plant is tolerant and hyperaccumulates Ni and Cd (Table 1).

Table 1. Metal accumulation and stress symptoms in chives.

Treatment (mM)	Time (days)	Harvest (number)	Contents, (mg/kg, d.w.)[1]		Stress symptoms
			Leaf	Root	
Ni 0.05	96	6	1110 ± 66	789 ±73	No
Ni 0.25	16	1	503 ± 35	1453 ±72	Wilting, GI[2]
Co 0.05	96	6	1002 ±59	726 ±125	Yellowing
Co 0.25	48	3	2643 ± 625	3000 ± 0	Yellowing, GI[2]
Cd 0.05	96	6	1166 ± 121	1314 ±123	No
Cd 0.25	64	4	2719 ± 723	2933 ± 116	GI[2]

[1] d.w. - dry weight; [2] GI - growth inhibition

There were no obvious stress symptoms over a period of 96 days (6 reharvests) at a concentration of 0.05 mM metal in the medium.

Comparison of Cd accumulation in chives to several other species reported in the literature (Table 2) showed that chives accumulated Cd to higher concentrations than *Brassica juncea*, *Allium sativum, Pisum sativum*, *Arabidopsis halleri, Lactuca sativa* or *Phragmites australis* (Table 2). The accumulation in *Allium schoenoprasum* leaves was at the level of accumulation in *Medicago sativa*. At the 0.25 mM Cd treatment, the metal concentration in *Allium schoenoprasum* leaves was higher than in the other species (Table 2).

Table 2. Cadmium accumulation in several plant species reported in the literature.

Plant species	Cd treatment µM	Medium	Time days	Contents (mg/kg, d.w.)[1]		References
				Leaf	Root	
Silene vulgaris	20.0	Liquid	21	1300[2]	>13000	Verkleij and Prast (1989)
Brassica juncea	17.8	Soil	14	104	-	Kumar et al. (1995)
Thlaspi caerulescens	63.2	Liquid	28	1290	3200	Brown et al. (1995)
Allium sativum	10000	Liquid	9	62.9	10100	Jiang et al. (2001)
Pisum sativum	40.0	Liquid	7	89	640	Dixit et al. (2001)
Medicago sativa	440.0	Soil	15	2002[2]	-	Peralta-Vedia et al. (2002)
Lactuca sativa	8.9	Liquid	16	16[2]	139	Ramos et al. (2002)
Phragmites australis	50.0	Liquid	21	77	3690	Iannelli et al. (2002)
Arabidopsis halleri	10.0	Liquid	7	157[2]	660	Bert et al. (2003)
Allium schoenoprasum (chives)	50.0	Liquid	14	199 (1166)[3]	1713	Khadka (2003)
Allium schoenoprasum (chives)	250.0	Liquid	14	745 (2719)[4]	4587	Khadka (2003)

[1] d.w –dry weight; [2] Shoot; - not reported; [3]in 6 re-harvests of the leaf of the same plants; [4]in 4 re-harvests of the leaf of the same plants

The accumulation of Cd in *Allium schoenoprasum* leaves in a single harvest was less than in *Thlaspi caerulescens* and *Silene vulgaris*. However, after 6 successive harvests *A. schoenoprasum* accumulated Cd at the highest level found in the cited references (Table 2). The accumulation of the metal in the root was also higher than in most other species.

The potential of chives for phytoremediation of heavy metals from contaminated water may become a handicap when grown for food on soil or water containing toxic metals. The accumulated metals may end-up on the consumers' plate. Thus it would be advisable to test the metal content in chives and other crop plants tolerant to heavy metals.

The chives model was good for the study of plant tolerance to heavy metals because of their richness in sulphur compounds. Some of them, such as cysteine and glutathione can serve as reductants or as scavengers to prevent oxidative damage of heavy metals. We have recently reported an increase in total SH-containing compounds in the root of chives under Cd stress conditions (Barazani et al., 2004). This was accompanied by a reduction in glutathione concentration in root, which raises the question about the identity of the newly synthesized SH compounds induced under the Cd treatment.

3.2 Iron Fortification of Food Plants

The other side of the coin of plant tolerance to heavy metals and phytoremediation is fortification of tolerant food crops with essential metals (Martinez-Navarrete, 2002). Iron deficiency is considered to be the commonest worldwide nutritional deficiency that affects approximately 20% of the world population. Women and children are especially at risk (http://www.who.int/nut/research1.htm). Promoting iron-rich foods as well as iron-fortified foods is necessary to reduce iron deficiency, as most of the people of the world get their iron from eating plant products. The tolerance of chives (*Allium schoenoprasum*) to heavy metals was reflected also in its ability to accumulate iron at approx. 800 mg/kg dry weight, when the medium was supplemented by 24 mg/L iron (data not shown).

4. DISCUSSION

Our preliminary findings show an almost linear correlation between iron concentration in the medium and its accumulation in the shoot. This taken together with the reported high concentration of vitamin C in chives, which improves iron availability in the diet, opens a new potential for promoting chives as an iron fortified health food.

Israel exports 1,500 tons of chives a year, representing 30% of the annual production. The remaining 70% is deemed inadequate for export. We propose that fortified chive production in Israel, especially the un-exportable yield, be dried, powdered and/or encapsulated and sold as a food additive/supplement. However, a critical question remains open concerning the nutritional quality of the fortified product in terms of the availability of the iron.

4.1 Metal Catalyzed Modification of Protein Amino Acids as Structural Signals

It is a well established fact that reactive oxygen species (ROS) can cause extensive cell injury, but in recent years it has become clear that they also play a role in plant metabolism, cellular signaling and defense (Davletova et al., 2005). Several researchers have reported the inactivation of enzymatic activities by the free radical forming ascorbate-Cu^{2+} system (Shinar et al., 1983; Nakamura and Stadtman, 1984; Golan-Goldhirsh et al., 1992 and others). This non-enzyme catalyzed reaction was site directed toward histidine residues of proteins and to a lesser extent toward methionine (Golan-Goldhirash et al., 1992). These results led to the proposed hypothetical 'structural signal' mechanism in response to metal stress (Figure 3).

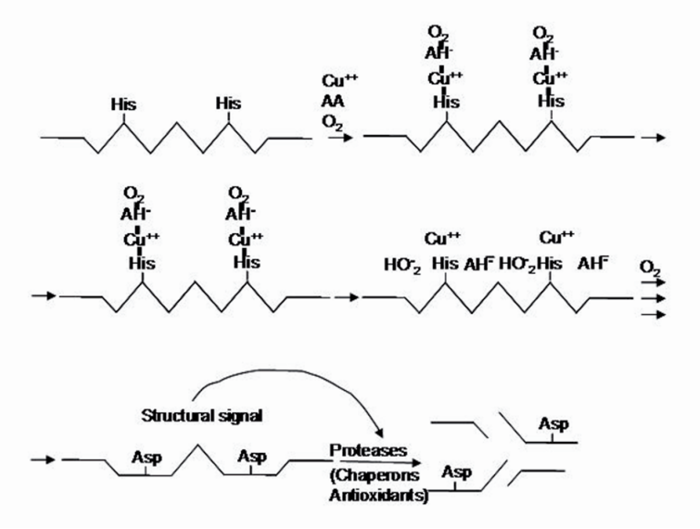

Figure 3. Schematic presentation of Ascorbate- Cu^{2+} catalyzed modification of histidine residues in protein leading to structural change and signaling to induce proteases, chaperons and antioxidative systems.

Higher specificity to histidine may be explained by the tight complex formation of Cu^{2+} with histidine compared to other amino acids (Golan-Goldhirsh et al., 1982). The proposed mechanism involves ascorbate anion combining with Cu^{2+} and O_2 forming a ternary complex on His, that dissociates to semidehydroascorbate radical, Cu^{2+} and the peroxy radical. Strong oxidants can be formed in the vicinity of the histidine residues of the protein leading to its modification into aspartate and possibly other degradation products (Golan-Goldhirsh et al., 1992). The modification of His leads to a structural change, which may mark proteins for degradation (Stadtman and Levine, 2003) and plausibly be a structural signal to induce proteases, chaperons and antioxidative systems in response to metal stress. Such a response can be envisioned as an "alarm" mechanism to metal excess and stress. It can thus be hypothesized that metal tolerant/hyperaccumulator plants would have more histidines in their proteins as a selective advantage in coping with metal stress. This hypothetical mechanism can also be partially verified *in vivo* by testing for induction of proteases, chaperons and antioxidative systems upon metal stress. More research is needed to understand the interactions of cupric ion and other metal ions with ascorbic acid and proteins and the role of ensuing structural changes of proteins on metal tolerance and homeostasis in plants.

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Assembled Plant and Microbial Technologies for Bioremediation of Pollutants

6.1. Ecoremediation. Cooperation between plants and soil microorganisms, molecular aspects and limits
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ECOREMEDIATION. COOPERATION BETWEEN PLANTS AND SOIL MICROORGANISMS, MOLECULAR ASPECTS AND LIMITS

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Abstract: In terrestrial ecosystems, higher plants are organized in two quite different spatial parts: a) a photosynthetic compartment, developed in the atmosphere, under light, and b) a soil compartment, specialized in the absorption of water and mineral salts, in the dark. An intense flux of water, of mineral salts and of organic compounds passes from the soil to the leaves. Small non-charged organic molecules with a medium lipophilicity can move passively in the transpiration stream, from soil water to the transpirating leaves. The composition of the soil solution is regulated by adsorption on the clay-humus complex and by exchanges with the microbial biomass. In the course of evolution, co-occuring microbes and plants have both developed, or maintained antitoxic properties allowing them to transform a large range of molecules, which are not among the group of essential compounds needed for microbial nor plant life. These antitoxic biochemical capabilities are generally not induced by the presence of a toxic property, but often only by foreign, unknown molecules. The case of phytoalexins seems to be an exception to this rule. Organic molecules reaching the soil are first submitted to microbial transformation, and plants absorb a mixture of molecules, of limited log P, either unchanged or partly transformed by microbes. During plant necrosis, the molecules which had been previously absorbed, and which are partly transformed by plant enzymes, are released into the soil and new transformations occur at this level, finally giving a complete mineralization. These mechanisms are illustrated by the case of two pesticides, atrazine and fipronil.

Key words: antitoxic function; atrazine; fipronil; remediation; safener

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1. INTRODUCTION

At the end of the XXth century, it was necessary to look for new techniques contributing to decrease or suppress heavy soil contamination resulting from earlier mining, industrial or military activities, or from accidents.

For this purpose, strategies relying on the use of living beings were developed, especially with soil microorganisms (bioremediation, landfarming...) (Felsot, 1996; Overcash and Pal, 1979) and with plants (phytoremediation) (Kruger et al., 1997). These strategies were sometimes based on the presence of a whole biomass, whatever the genus or the species involved, and supposing that the presence of the contaminant or of the contaminant mixture would play the role of a screen selecting the fittest species in this situation. However, there was a marked trend in scientific research to select or create, through genetic engineering, species or strains with noteworthy biochemical capabilities supposed to allow a quicker and better remediation (Shelton et al., 1992). This trend, using biochemical diversity, was initiated by scientists working on ecology, who showed that specific plants and microbe strains were growing spontaneously in heavily contaminated places with concentrations of xenobiotics just below the fully abiotic ones.

From a practical point of view, some bioremediation strategies were impressive successes and are now commonly used. It is the case of reed beds for instance, used to purify water. It is also the case, in agricultural areas, for soil restoration after pesticide treatments and before new cultures. This was extensively studied for numerous active ingredients and over years, giving a huge number of experimental results in the fields of agronomy, physiology, ecology, biochemistry, genetics, chemistry, so that it represents most of the knowledge about the dissipation of organic xenobiotics in the soil of terrestrial ecosystems.

The purpose of this report is to show the general features of xenobiotic remediation resulting from the cooperation between plants, soil, water and microbial biomass in the field.

2. RESULTS

2.1 Exchanges in a Terrestrial Ecosystem

2.1.1 Inside Terrestrial Plants

The plants adapted to terrestrial life are composed of two parts: one is located inside the atmosphere, and is the place where organic matter synthesis occurs, in the presence of light, CO_2 and H_2O.

As the atmospheric water pressure is often low, an important loss of water takes place at this level. A water absorption in the soil makes up for it and induces a powerful xylem sap stream.

The second part in such plants develops inside the soil and depends on the organic matter produced in the first part. A second water movement, from the aerial parts to the roots and to all the expanding organs occurs inside the phloem sieve tubes, distributing organic soluble matter (Figure 1).

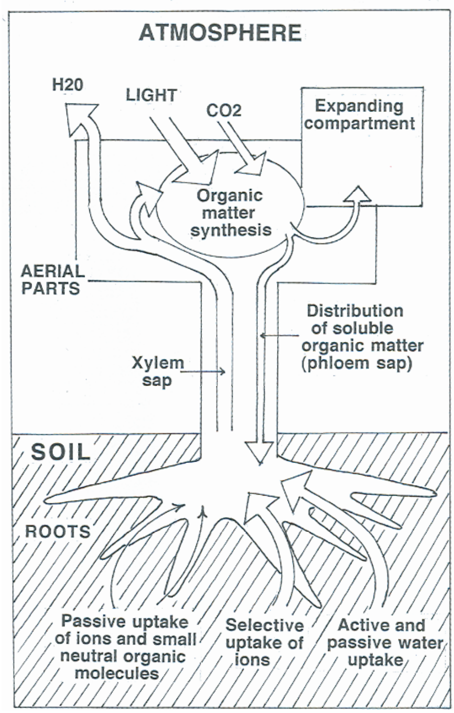


Figure 1. Scheme illustrating the presence of two compartments in plants adapted to terrestrial life.

The two types of water movements (upwards in the xylem vessels; downwards in the phloem sieve tubes) can be the origin of xenobiotic small molecules displacements. Depending on transpiration, the first role of the plant cover is hence to accumulate the substances added to soil inside the aerial parts via the xylem sap stream. As a consequence, it prevents or limits their transfer from the soil surface to the groundwater.

2.1.2 The Consortium Plant/Water/Clay-humus Complex/Microbial Biomass

In nature, the plant cover produces a high amount of organic matter which gives access to the formation of humus, a complex, water-insoluble, negatively-charged polymer (Figure 2). Its mixture with clay gives the clay-humus complex (C.H.C.) to which a large series of ions and small organic molecules can be bound. This binding is measured with the Kd parameter expressing the concentration ratio between soil and surrounding water under standard conditions. Moreover, for organic molecules, it was shown that binding mostly depends on the organic part of the C.H.C. at least for a carbon concentration higher than 1 % in the complex (Green and Karickhoff, 1990). In this case, the binding properties of the C.H.C. may be expressed with the Koc parameter, which is the ratio between the apparent concentration of the studied xenobiotic in the organic part of the C.H.C. and surrounding water. C.H.C., in the well-oxygenated upper soil layer, harbors an intense microbial life. The corresponding microbial biomass is composed of several groups of species having complementary nutritional characteristics and being able to rapidly adapt when surrounding conditions change.

Under in vitro conditions, one can study the plant activities separately, when growing them on artificial liquid media. In nature, the situation is quite different and a constant exchange occurs between plants, water, C.H.C. and microbial biomass (Figure 3). Under such conditions, the degradation of unwanted xenobiotic molecules can never be the result of plants alone and « ecoremediation », represents the cooperative action of the four parts that constitute the soil-plant system.

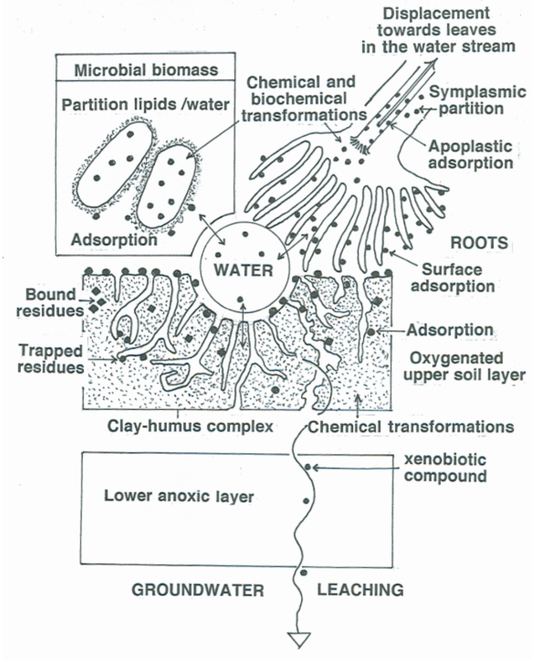


Figure 2. The consortium plant/water/clay-humus complex/microbial biomass.

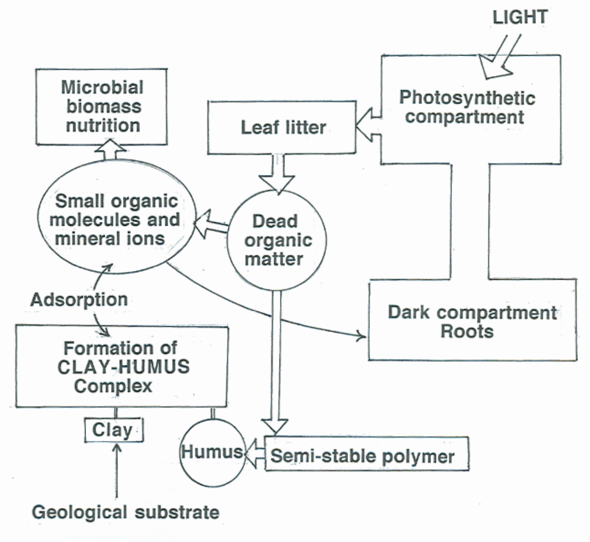


Figure 3. The plant-dependent formation of the clay-humus complex.

2.2 What is an Antitoxic Function in Plants and Soil Biomass?

2.2.1 Microorganisms

The full degradation of an organic xenobiotic compound by a soil microbial biomass can be demonstrated in the laboratory, using the ^{14}C labelled compound and measuring ^{14}C emission. Two pathways having a different physiological significance may be distinguished : a) A true metabolic pathway in which the degrading microorganisms obtain C, N or energy from the xenobiotic. In this case, this compound can be considered as a nutrient. b) A « co-metabolism » pathway, in which microbes are able to transform the xenobiotic without deriving any nutrient or energy from the process (Bollag and Liu, 1990). In that case, the co-metabolism activity may be considered as an antitoxic function if the compound shows toxic properties against one or several species among the microbial biomass.

Clear co-metabolism degradation has been described for instance in the case of the herbicide alachlor (Novick et al., 1986). True metabolism has been shown for atrazine, with species using it as sole C and N sources (Mandelbaum et al., 1993).

2.2.2 Plants

In animal beings, especially in mankind, a physiological function allowing elimination of several foreign undesirable molecules has been well-established. It generally consists in transforming the product in order to give it a reactive site (phase I) allowing the formation of a hydrophilic derivative (phase II) which can be eliminated with urine or feces (phase III). This function seems of primary importance in the case of heterotrophic beings, especially predators. The case of plants is not so clear by far. They lack any clear-cut general excretory system and, in contrast, they present a large apoplastic space, either relatively lipophilic (lignin, oil droplets, waxes) or hydrophilic (vacuole), where numerous compounds might be accumulated with far less danger than in the symplasm. The toxic effect on plant cells can be obtained through several, quite different biochemical mechanisms which are intensively explored for herbicide discovery. Some of these effects are fairly selective of plants (Inhibition of D1 protein activity, inhibition of plant tubulin polymerization, carotenoid biosynthesis alteration ...) (Tissut and Séverin, 1984). Others disrupt a general mechanism of life in eukaryotic or even prokaryotic cells. It is the case, for instance, of highly lipophilic compounds which are very common among industrial waste products. Those compounds may or not have uncoupling properties. The partition process powerfully concentrates them in biological membranes. For instance, in the case of pentachlorophenol (log P close to 4), acting on isolated mitochondria, a concentration of 0.01 mM in the medium leads to a membrane concentration reaching 0.1 M (Albertin et al., 1991). Such a membrane concentration, even for products without specific uncoupling action, leads to chaotropic effects. This was demonstrated namely in the case of synthetic monoterpenoids (Balland et al., 1997). The most common chemical family of synthetic uncouplers is that of substituted phenols (2,4-dinitrophenol, pentachlorophenol...) which have been extensively used as general biocides (Tissut and Séverin, 1984) and thus remain major pollutants. Plants have metabolic competences for these products (Schmidt et al., 1985) but only as far as their energetic metabolism is not deeply altered. This point illustrates the fact that plant defence mechanisms can be effective only below a threshold rate of xenobiotic penetration.

Some plant species are now known to possess very sophisticated biochemical mechanisms able to transform and metabolize different xenobiotic compounds in the same general way as animals do (Figure 4). Most of our knowledge on this point is coming from agronomy and pesticide science. To know that a cultivated plant possesses a specific resistance or tolerance to a weed-killer is of first agronomical and economical importance.

One of the most commonly encountered mechanisms of phase I metabolization is oxidation. It can be catalyzed by phenolases, peroxidases, peroxigenases or P-450-mixed function oxidases (Durst, 1991a). P-450 cytochromes represent a multigenic family of homoproteins, having a constantly present part which is a protoporphyrin IX with an iron atom, able to bind molecular oxygen. Another part of these compounds is a lipophilic protein. Its structure may differ widely from one type of P-450 to the others (M.W. from 43 to 60 kDa). Xenobiotics bind to this protein. P-450 are localized in endoplasmic reticulum. A very large number of P-450 isoforms has been described in the plant kingdom. Several hundreds of substrates are transformed more or less specifically by P-450, most of which are xenobiotics.

The panel of xenobiotic substrates able to be transformed by the P-450 isoenzymes in one plant is far from being well-known at the present time. However, it was acknowledged that this panel deeply changes from one species to the others. It was first supposed that P-450 isoforms were enzymes with a low substrate specificity. It seems now that this was not true and that most isoforms are acting only on one chemical family of substrates. The same hypothesis seems likely in the case of glutathion-S-transferases (GST) (Marrs, 1996). Hundreds of GST isoforms have been described in plants, having different substrate specificities. For instance, in corn, two types of isoenzymes having chloro-S-triazines as substrates have been described, owning to group I and group IV GSTs (Wiegand et al., 1986; Grove et al., 1988). Others isoforms are present, transforming other substrates (Cl-1,diNO$_2$-2,4 benzene, chloroacetamides).However, when comparing the rate of substrate transformation by corn GSTs, it is thousand times higher for CDNB than for atrazine.

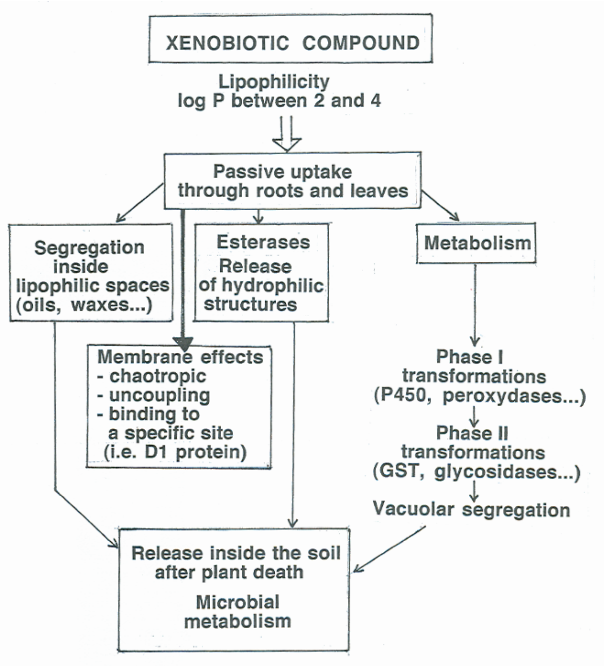


Figure 4. Fate and possible effects of lipophilic xenobiotics inside plants.

As a whole, it seems that most plants have metabolic capabilities for foreign, eventually toxic molecules. The substrate specificity of these capabilities deeply differs from one species to the others.

As the antitoxic function was probably not a strongly limiting factor during the evolution-adaptation processes, but as it may represent an advantage in specific circumstances, it seems that a global metabolic capability has been maintained during evolution, but with a wide biochemical diversification from one species to the others.

A full phytoremediation process would lead to recycle the atoms constituting the xenobiotic into organic compounds normally present in living plants. The rate of such a transformation is likely to be very low as it is easy to see that the first metabolites of xenobiotics always accumulate in plants. This can be understood when taking into account several features of cell activities.

1. Lipophilic pollutants are the normal substrates for phytoremediation (Hydrophilic ones cannot enter the plant). Partition occurs inside plants and the lipophilic compound accumulates, either into lipophilic aposplastic spaces (lignified cell-walls, cuticles, waxes or storage oils) or into biological membranes, most of which are unable to metabolize the compound. This accumulation is the first condition for a high-rate-transfer into food chains. All these partition processes are reversible but at a low rate (Raveton et al., 1999).
2. A reactive biological membrane such as ER could lead to transfer to Golgi vesicles and exocytosis into cell-walls, possibly giving bound residues.
3. The products of phase I transformation could be hydrophilic enough to be mostly accumulated inside the semi-apoplastic space corresponding to the vacuole. They can therefore escape further structural changes. This is the case of hydroxyatrazine in corn. This vacuolar segregation also hinders the further transformations of hydrophilic conjugates, although transformation of the hydrophilic moiety (sugars, amino-acids) could occur, as was shown in corn for glutathion. In wheat, hydrolysis of vacuolar esters was also demonstrated.

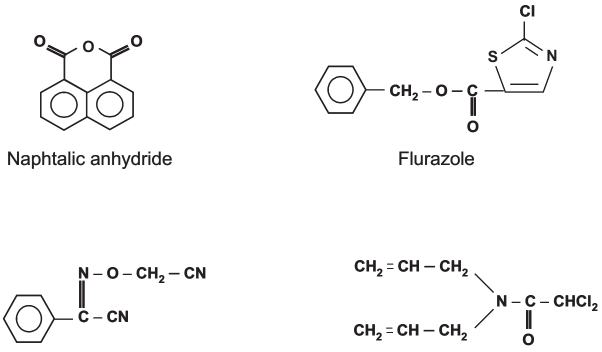
As a whole, xenobiotic metabolism in plants gives transformation products coming either from phase I or phase II or I + II, which mostly accumulate during phase III without any rapid transformation.

Careful measurements of the turnover of these derivatives remain to be carried out and are probably difficult, due to the massive dilution of these substances in the huge amount of natural products.

A tightly programmed antitoxic function in plants would imply that the metabolic capabilities against xenobiotics were induced specifically by the presence of such a product .In fact, the biosynthesis of enzymes involved in xenobiotic transformation is seldom the result of a constitutive regulation but is often due to external stimuli, either physical or chemical. As a matter of fact, the metabolic defence mechanisms can be induced by several types of external stimuli (wounding, light, biological aggression, heavy metals, chemicals...). Chemical induction is chemically selective. The xenobiotic molecules which were shown to induce the expression of enzymes (mostly P-450 or GST) involved in the detoxification of other xenobiotics were named « safeners » (Wiegand et al., 1986).

Durst (1991b) has shown some stimuli involved in P-450 induction in plants.

Very interestingly, classical safeners acting on enzyme induction in animals and man (phenobarbital, aminopyrine, clofibrate) are equally efficient in several plants. Some of the most commonly known safeners used in agriculture are shown in Figure 5.



Naphtalic anhydride

Flurazole

Cyometrinil

Dichlormid

Figure 5. Structure of safeners commonly used for plants.

The overview of the biochemical metabolizing properties of several plant species for xenobiotic compounds shows a high diversity in the plant kingdom. Moreover, it is impossible not to be struck by the analogy existing between xenobiotic metabolism and common secondary metabolisms such as that of flavonoids in plants: the p-hydroxylation of a phenolic ring is controlled by inducible P-450; formation of conjugates is carried out prior to vacuolar excretion; terminal compounds are accumulated. These secondary plant metabolisms appear therefore as typical phase I, II and III processes. Moreover, most of our knowledge about glycosyltransferases and active transport into the vacuole was obtained when studying these compounds and not xenobiotics (Leah et al., 1992).

As a whole, it seems that the antitoxic functions of plants were maintained throughout evolution but with a large diversification of the enzyme substrate specificities. These biochemical capabilities can be understood in relation to the discovery by plants of phytotoxins as instrumental in competing with other species (Figure 6). They differ from the fundamental metabolisms always present in the whole plant kingdom, and from the metabolisms which are characteristic of the plant model adapted to terrestrial life.

They seem, in fact, not to be induced by the expression of a toxic effect but by the presence of different types of unknown, foreign molecules. However, some of them may be related to stress reactions and require further studies. As shown in Figure 6, the substrate specificities of these catabolic isoenzymes (GST, P450…) are quite different from one plant to another. The truly antitoxic function that these isoenzymes might play in one plant species against a toxic compound seems to be obtained by chance. This has been unambiguously demonstrated by several scientific reports about selective herbicides (Tissut and Séverin, 1984).

At the level of an ecosystem, its ecoremediation potential for organic compounds is the sum of all the catabolic capabilities of each living species present in it. This global potential is now supposed to cover a large range of substrates specificities and thus to be an efficient ecoremediation tool.

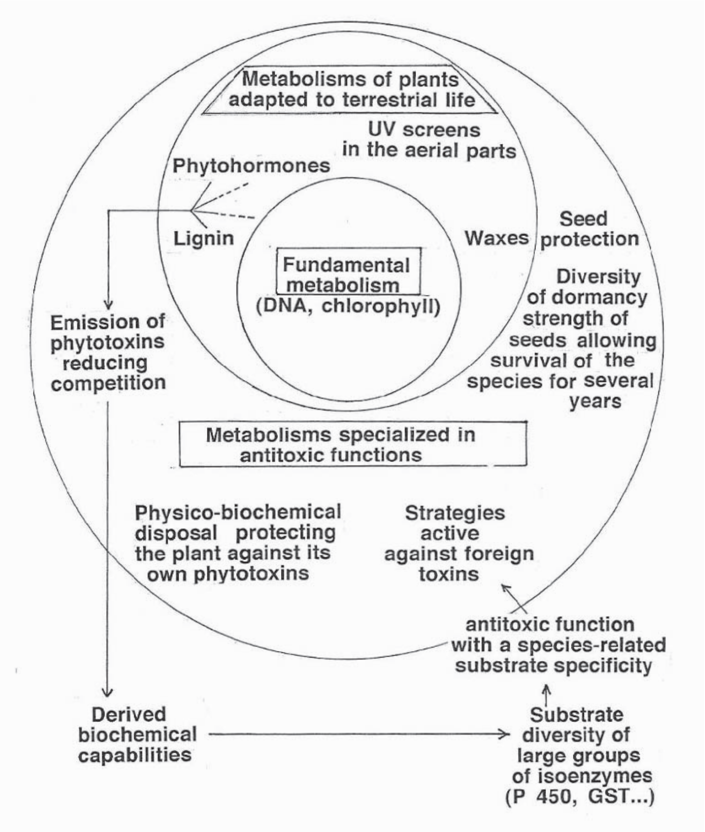


Figure 6. Adaptative metabolism for phytotoxins and antitoxic functions in plants.

2.3 Two Examples of Ecoremediation in Agronomy

2.3.1 The Herbicide Atrazine (Figure 7)

Our lab studied qualitative and quantitative dissipation of this molecule for three years in corn culture treated with 1 kg/ha active ingredient (a.i.). After 5 months, (Figure 8), the amount absorbed and transformed by corn was approximately 50g, that fully transformed in the soil was between 100 and 300g. A remnant amount composed of the active ingredient and the first metabolites reached 400g. The part supposed to have been leached into the groundwater weighed between 50 and 300g (Tasli et al., 1996).The limited role played by corn in atrazine dissipation (5 %) under agronomic conditions was explained by several points: 1. The degradation potential of the soil microflora was high and present in each point of the upper soil layer, in contrast with the plant cover (80000 plants/ha). 2. Atrazine was bound to the C.H.C. with a Kd close to 280. The amount of atrazine dissolved in soil water was therefore low. Root uptake occurred mostly from this solution 3. In the treatment mixture, atrazine was present under a microcrystalline solid form, which remained on the soil surface for weeks and dissolved in soil water only slowly.

Other labs studied the rate of atrazine degradation in various field conditions (i.e. Erickson and Lee, 1989). Several months were generally necessary for half-life. The herbicidal potential for adventicious plants had fully disappeared after one year in France. The first steps of metabolization (N-dealkylation, hydroxylation, glutathion conjugation) momentously lowered herbicidal efficiency.

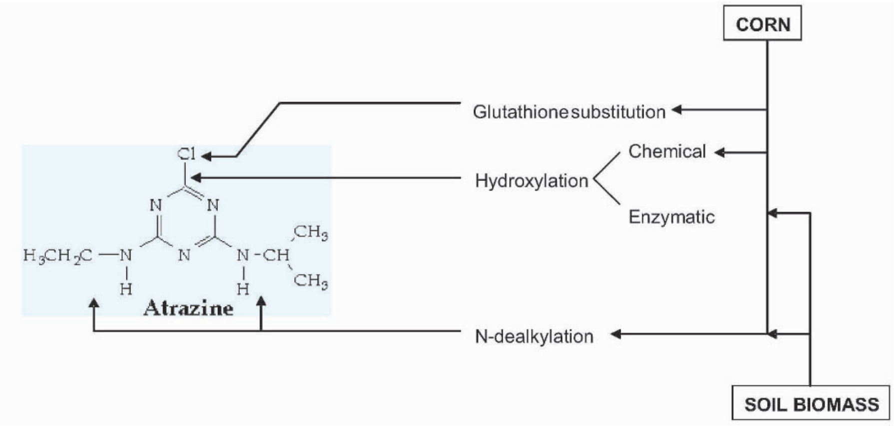


Figure 7. Transformations of the herbicide atrazine in corn and soils.

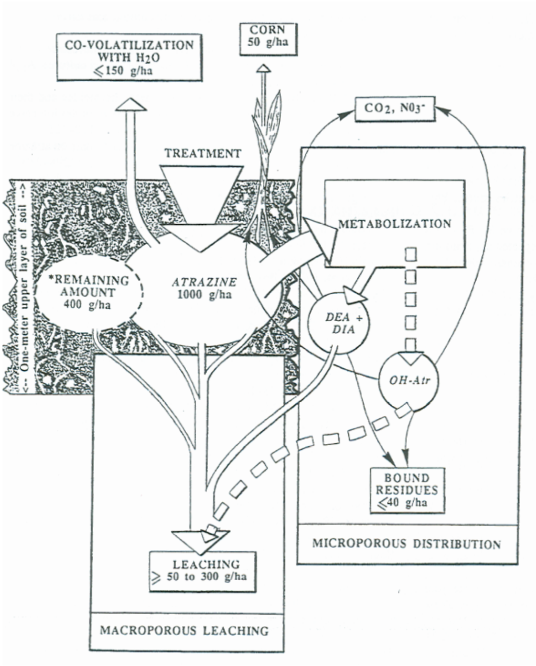


Figure 8. Quantitive features of atrazine transformations in soils.

2.3.2 The Fipronil Insecticide

This insecticide of the phenylpyrazole family acts on the chloride channel of the nervous system, mostly in insects (Colliot et al., 1992; Cole et al., 1993; Blooomquist, 2000). It is used as seed coating against wireworms in corn, beet and sunflower cultures.

Under these agronomic conditions, the percentage absorbed by plants is less than 1% of the treatment although the potential ability of plant absorption is much higher. The limitation is due 1) to the difficult passage from the solid form in the coating into the soluble form, and 2) to tight binding to the C.H.C. Fipronil is without noticeable toxic activity against plants and microorganisms. However, they metabolize the a.i. at a medium rate, with a half-life of several months. Sunflower and microbial biomass degrade it following a similar scheme (Figure 9). An additional photochemical reaction occurs, under light, leading to a stable derivative (Bobe et al., 1997; Hainzl ad Casida, 1996), with a CF3 substituant in the 4-position. All the lipophilic derivatives where the pyrazole ring is maintained have a high insecticidal activity. A significant loss of it is obtained with the 3-$CONH_2$ derivative which is hydrophilic. After heterocycle cleavage, the aniline derivatives show an insecticidal activity at 0.1mM which is probably due to non-specific membrane effects. Under such agronomic conditions, one may conclude that the insecticidal efficiency of the a.i. against wireworm is warranted but ecoremediation is difficult.

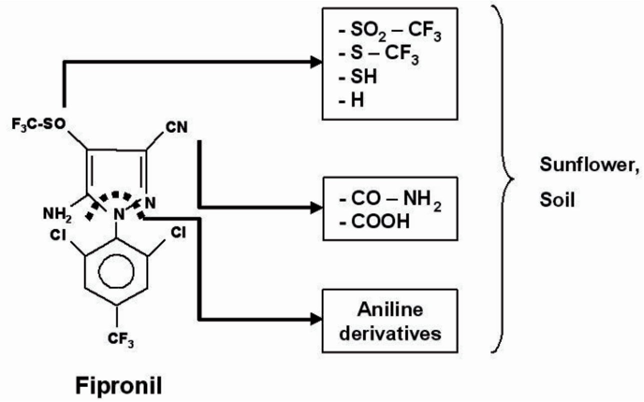


Figure 9. Biological degradation pathaways for fipronil.

2.3.3 Discussion

The association of plants with microbes for bioremediation of chemically contaminated areas has demonstrated its efficiency in numerous cases. Plants bring about two main advantages: 1)They create a rhizosphere effect, 2) Through transpiration, they prevent or limit downward leaching of the xenobiotics. Moreover some of them can transform the xenobiotics they have absorbed.

In the case of heavy contamination, ecoremediation will work only if plants and microbes remain alive. Under less drastic conditions, ecoremediation activities seem to be an appropriate tool with relatively low cost and compatible with agricultural practices.

In wild areas, all of which are now submitted to a chronic, complex, atmospheric contamination, ecoremediation probably works spontaneously. A better knowledge of this phenomenon requires an intense scientific effort in order to understand if, in each ecosystem, this biochemical potential allows to maintain an acceptable equilibrium or not and if better practices may be conceived.

In agrosystems, fifty years of experiments on pesticides demonstrate that full degradation of xenobiotics at low concentration (less than 1 to 4 kg/ha) is seldom obtained readily. It generally requires several months and is followed by substance displacements from the treated area to deeper soil, to water and to the atmosphere. However, landfarming techniques allow increasing the yield of ecoremediation in such systems if necessary.

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ANAEROBIC DEHALOGENATION OF HALOGENATED ORGANIC COMPOUNDS: NOVEL STRATEGIES FOR BIOREMEDIATION OF CONTAMINATED SEDIMENTS

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Abstract: Remediation of sediments contaminated with toxic chemicals is one of the greatest challenges for restoration of estuaries. Halogenated organic compounds constitute one of the largest groups of environmental pollutants and their use has resulted in widespread dissemination and environmental contamination, with freshwater, estuarine and marine sediments as significant sinks. Consequently, the management of sediments contaminated with toxic organic compounds, including polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), pesticides and brominated flame retardants, is a major problem with far-reaching economic and ecological consequences. Anaerobic dehalogenating populations appear to be abundant in estuarine and marine sediments and many chlorinated and brominated aromatic compounds are readily dehalogenated, potentially leading to complete degradation and mineralization. For example, dechlorination of chlorinated dioxins and dibenzofurans is readily promoted in sediments from several sites. The biodegradability of organohalides is affected by available electron donors and acceptors, and the dehalogenating microbial populations active in different redox zones are distinct. Co-amendment with halogenated analogues enhanced dechlorination of spiked PCDD/Fs in estuarine sediments under a variety of conditions. Enhancement of microbial dehalogenation is an attractive remediation alternative that could potentially detoxify sediments and avoid the problematic redistribution of contaminants that is associated with dredging. Microbial reductive dechlorination is an important environmental process because it has the potential of decreasing the toxicity of PCDD/Fs if lateral chlorines are removed. These fundamental studies are providing an understanding of how dehalogenation processes are incorporated into a global

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"halogen cycle" and are serving as a base for developing new methods for bioremediation of contaminated sediments.

Key words: organohalide pollutants; contaminated sediments; anaerobic dehalogenation; polychlorinated dibenzo-*p*-dioxins; halogenated flame retardants

1. INTRODUCTION

Remediation of sediments contaminated with toxic chemicals is one of the greatest challenges when restoring estuaries and coastal salt marshes. Halogenated organic compounds constitute one of the largest and most problematic groups of environmental pollutants. These compounds are integral to a variety of industrial applications, including use as solvents, degreasing agents, biocides, pharmaceuticals, plasticizers, hydraulic and heat transfer fluids, intermediates for chemical synthesis, flame retardants and numerous other industrial functions. The majority of these compounds are chlorinated, but brominated, fluorinated and iodinated compounds are also used in industrial applications (Häggblom and Bossert, 2003). Their use has resulted in widespread dissemination and environmental contamination, with estuarine and marine sediments as significant sinks. Consequently, the management of marine and estuarine sediments contaminated with toxic organic compounds, including polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), PCBs, pesticides and brominated flame retardants, is a major problem with far-reaching economic and ecological consequences.

Microbial degradation is one of the key factors that determines the ultimate fate of organohalides in the environment, with cleavage of the carbon-halogen bond being one of the critical steps. Microbial degradation requires the presence of enzymes that cleave this bond under physiological conditions and microorganisms have evolved a variety of metabolic strategies for cleaving the carbon-halogen bond (for reviews, see Häggblom, 1992; Häggblom and Bossert, 2003; Holliger et al., 2003; Löffler et al., 2003). Dehalogenation reactions comprise different strategies, where organohalides serve either as electron donors (and carbon sources) or as electron acceptors. In anaerobic sediments the most important process is one in which the organohalide serves as an alternate electron acceptor for anaerobic respiration, termed dehalorespiration. Microbial degradation of organohalides in anoxic sediments is widely observed (Häggblom et al., 2000, 2003), although the availability of suitable electron donors and acceptors affects the biodegradability of organohalides. Anaerobic dehalogenation is influenced by the microbial communities active in different redox zones.

Enhancement of microbial dehalogenation is an attractive remediation alternative that could potentially detoxify sediments. Furthermore, remediation of organohalide-contaminated sediments *in situ* could avoid the problematic redistribution of contaminants that is associated with dredging and would decrease the cost of sediment management. However, application of *in situ* bioremediation of organohalide-contaminated marine and estuarine sediments has been limited by lack of fundamental knowledge about the microorganisms responsible for contaminant degradation, including anaerobic reductive dehalogenation, the first step required for the ultimate complete degradation of highly halogenated compounds. Application of bioremediation to organohalide-contaminated sediments is also limited by the low bioavailability of these compounds and the inability to effectively stimulate biodegradation. The lack of knowledge about the microorganisms responsible for dehalogenation of PCDD/Fs and PCBs in particular, also presents a limit on engineered manipulation of estuarine sediments. Detailed information on the dehalogenation process is needed to provide strategies for remediation of contaminated sediments by enhancing dehalogenation of organohalogen pollutants *in situ* and/or in conjunction with sediment caps.

2. IDENTIFICATION OF DEHALOGENATING POPULATIONS IN MARINE SEDIMENTS

Microbial degradation of organohalides in anoxic sediments is widely observed. In marine and estuarine sediments the interactions with other competing respiratory electron acceptors will have a direct impact on the activity of the microbial community and biodegradability of organohalide compounds (Bossert et al., 2003; Häggblom et al., 2003). The prevailing electron accepting processes that participate in dehalogenation are determined not only by the availability of electron accepting species, but also by the concentration of electron donors, especially hydrogen, in the environment (Fennell and Gossett, 1998, 2003). Competition for hydrogen (or other simple organic electron donors) is key in determining the succession of microorganisms and redox processes.

Reductive dehalogenation can proceed simultaneously with other dominant electron-accepting processes, with further degradation of the dehalogenated product coupled to methanogenesis, sulfate reduction, or iron(III) reduction (Steward et al., 1995; Häggblom, 1998; Häggblom et al., 1993; Häggblom and Young, 1995; Boyle et al., 1999a, 1999b; Knight et al., 1999, 2003; Monserrate and Häggblom, 1997; Sun et al., 2000, 2001). The dehalogenating populations are distinct under different redox conditions, e.g., sulfidogenic, iron-reducing or methanogenic (Knight et al., 1999; Häggblom et al., 2000; Rhee et al., 2003; Fennell et al., 2004a). Differences in substrate specificity, differences in apparent rate of dehalogenation, and unique community profiles indicate that distinct microbial populations are responsible for reductive dehalogenation under different dominant terminal electron accepting conditions (Knight et al., 1999; Häggblom et al., 2000, 2003).

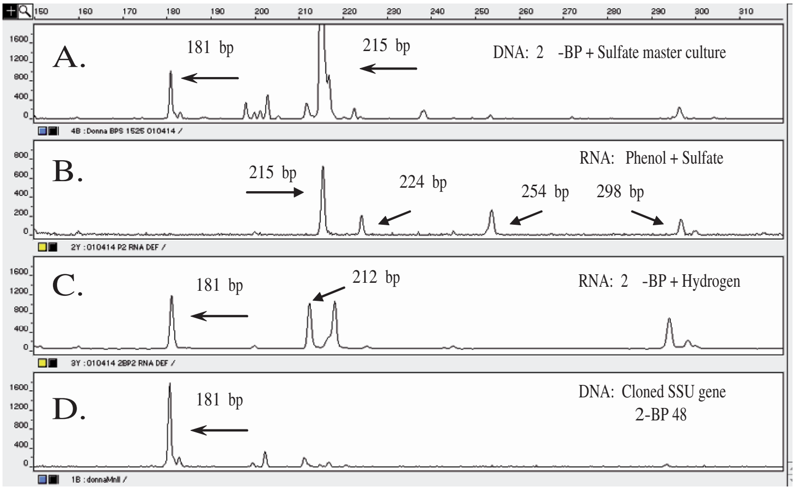

Figure 1. Comparison of T-RFLP analysis of genomic DNA from master culture 16S rRNA gene (A), reverse transcribed 16S rRNA from knock-out cultures phenol + sulfate (B), 2-bromophenol + hydrogen (C) and cloned 16S rRNA gene of 2BP-48 (from Fennell et al., 2004a).

We have pursued the characterization of the structure and diversity of microbial food webs that are active during dehalogenation in anaerobic estuarine and marine sediments in order to identify the dehalogenating organisms. We have applied an assay using a starved consortium (low ribosome content culture) fed a suite of selective substrates (Fennell et al., 2004a). The newly synthesized 16S rRNA was then analyzed using terminal-restriction fragment length polymorphism (T-RFLP) analysis of reverse transcribed rRNA as a means for assigning a metabolic role to the different members of the sulfidogenic 2-bromo-phenol-dehalogenating consortium. Those organisms that became active after substrate feeding were recognized as distinct peaks in the electropherogram and could be identified based on data from clonal libraries. Direct terminal restriction fragment length polymorphism fingerprinting of ribosomes in the various subcultures (fed different substrate combinations) indicated that phylotype 2BP-48 (a *Desulfovibrio*-like sequence reported previously; Knight et al., 1999) was responsible for the dehalogenation of 2-bromophenol (Figure 1). A stable co-culture was established containing predominantly 2BP-48 and a second *Desulfovibrio* species capable of mineralizing 2-bromophenol coupled to sulfate reduction. Strain 2BP-48 in the co-culture could couple reductive dehalogenation to growth with 2-bromophenol, 2,6-dibromophenol, or 2-iodophenol with lactate or formate as an electron donor. Interestingly, this strain is capable of simultaneous sulfidogenesis and reductive dehalogenation in the presence of sulfate.

The use of functional genes (Rhee et al., 2003) to elucidate dehalogenating microorganisms in consortia or natural environments is an alternative to the indirect method using ribosomes. Recently, several membrane bound reductive dehalogenases (RDH) have been purified and characterized (see Holliger et al., 2003). Several sets of degenerate PCR primers to amplify RDH gene fragments were designed and putative RDH genes were cloned and identified from estuarine and marine environments (Rhee et al., 2003; Ahn et al., 2003, unpublished; Figure 2).

The amino acid sequences deduced from the C-terminal region of the enzymes were compared and showed high similarity. However, several of these putative RDH genes were linked to transposon-like sequences and may, in fact, be pseudogenes (Rhee et al., 2003). Thus, additional assays must be done at the mRNA level to identify expressed RDH genes in natural samples in order to monitor dehalogenating populations.

We have used 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) and 1,2,3,4-tetrachlorodibenzofuran (1,2,3,4-TeCDF) as model contaminants to examine the potential for microbial reductive dechlorination of PCDD/Fs and to develop highly enriched cultures for determination of the identities of the dechlorinating organisms (Vargas et al., 2001; Fennell et al., 2004b; Ahn et al., 2005). We have characterized the PCDD/F dechlorinating capability of native dehalogenating bacteria from using a variety of electron donor and halogenated co-amendments (Ahn et al., 2005). PCDD dechlorination was observed in different freshwater, estuarine and marine sediments, suggesting the ubiquitous nature of these bacteria. 1,2,3,4-TeCDD was sequentially dehalogenated, initially in the lateral position, leading to the formation of 2-monoCDD (Figure 3.) While reductive dechlorination of PCDDs occured in sulfate-rich environments, the rates of dechlorination were slower than those under methanogenic conditions. The pathways may also be different, possibly indicating the stimulation of phylogenetically different organisms (Ahn et al., unpublished). There have been many reports that the presence of

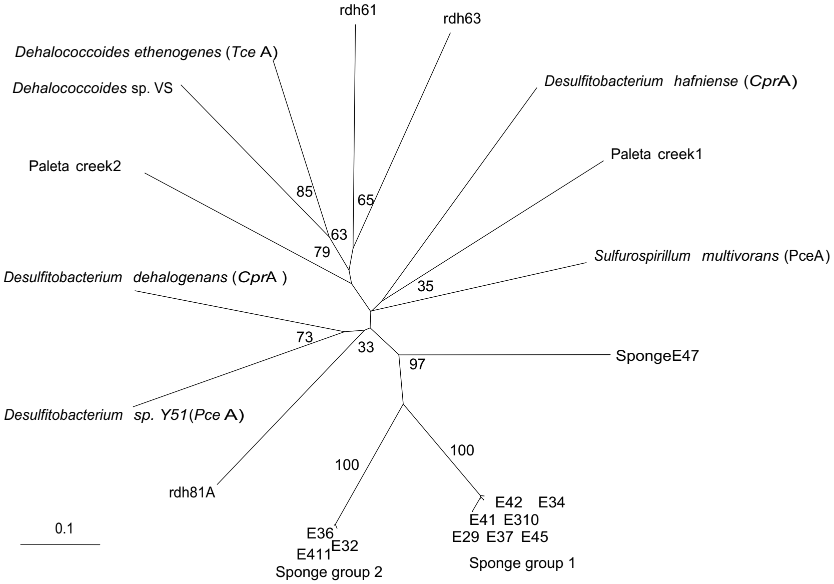

Figure 2. Phylogenetic tree of deduced partial amino acid sequences of reductive dehalogenases. Reference bar indicates 10 amino acid exchanges per 100 amino acids.

sulfate inhibits reductive dechlorination of PCDDs (Vargas et al., 2001) and PCBs (Alder et al., 1993; Zwiernik et al., 1998). It is at the same time interesting to note that some of the dehalogenating organisms that have been characterized are themselves sulfate reducers (DeWeerd et al., 1991; Boyle et al., 1999c; Ahn et al., unpublished).

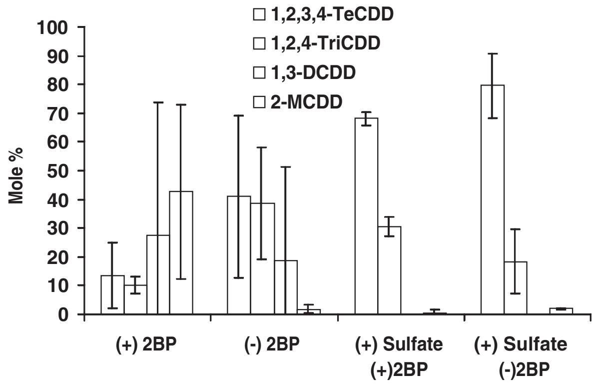

Figure 3. Mole % 1,2,3,4-TeCDD and its dechlorination daughter products in Paleta Creek sediment under methanogenic and sulfidogenic conditions with and without the addition of 2-bromophenol (from Ahn et al., 2005).

3. *DEHALOCOCCOIDES ETHENOGENES* DECHLORINATES DIVERSE CHLORINATED AROMATIC POLLUTANTS

Dehalococcoides ethenogenes strain 195 dechlorinates tetrachloroethene to vinyl chloride and ethene and its genome has been found to contain up to 17 dehalogenase gene homologues (Seshadri et al., 2005) suggesting diverse dehalogenation potential. Detection of multiple dehalogenase homologs in other strains (Hölsher et al., 2004) provides further evidence for the metabolic versatility of this phylogenetic group. Within the Chloroflexi, which includes both *Dehalococcoides* spp. and other yet to be described genera, there appears to be several strains with the ability to reductively dechlorinate PCBs and other halogenated compounds as electron acceptors (Wu et al., 2002a; Bunge et al., 2003; Watts et al., 2001; Miller et al., 2005).

We have demonstrated that *D. ethenogenes* strain 195 has the potential to dechlorinate many different types of chlorinated aromatic compounds, in addition to its known chloroethene respiratory electron acceptors (Fennell et al., 2004b). *D. ethenogenes* strain 195 dechlorinated 1,2,3,4- tetrachlorodibenzo-*p*-dioxin to a mixture of 1,2,4-trichlorodibenzo-*p*-dioxin and 1,3-dichlorodibenzo-*p*-dioxin (Figure 4). 2,3,4,5,6-Pentachlorobiphenyl was dechlorinated to 2,3,4,6- and/or 2,3,5,6-tetrachlorobiphenyl and 2,4,6-trichlorobiphenyl. 1,2,3,4-Tetrachloronaphthalene was dechlorinated primarily to an unidentified dichloronaphthalene congener. Results obtained after re-amendment of cultures with PCE suggest that the dehalogenation observed was not necessarily dependent on on-going PCE dehalorespiration since no concomitant increase in the rate of chloroaromatic dechlorination was observed.

The ability of *D. ethenogenes* strain 195 to dehalogenate such a diverse array of organohalides is significant given the prevalence of *Dehalococcoides*-like organisms in the environment. Bunge et al. (2003) have shown that *Dehalococcoides* sp. strain CBDB1, which was originally cultivated on trichlorobenzene (Adrian et al., 2000), dehalogenates and sustains growth on chlorinated dioxins. Highly enriched cultures containing strains DF-1 and o-17, bacteria distantly related to *Dehalococcoides*, are able to dehalogenate chlorinated biphenyls (Wu et al., 2002a; Cutter et al., 2001) and DF-1 also dechlorinates the chlorinated benzenes (Wu et al., 2002b). Aside from these reports, little is known about the anaerobic microorganisms that mediate reductive dechlorination of PCDD/Fs, which is required to initiate complete degradation of highly chlorinated congeners (Toussaint et al., 1998; Gruden et al., 2003), or how to enhance the growth of these organisms under field conditions. Microbial reductive

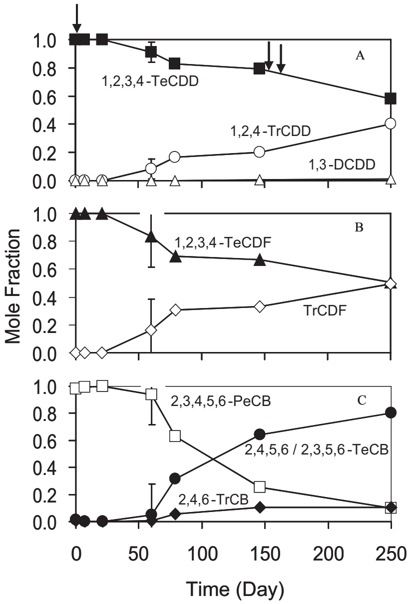

Figure 4. Dechlorination by *Dehalococcoides ethenogenes* strain 195 of (A) 1,2,3,4-tetrachlorodibenzo-p-dioxin (B,) 1,2,3,4-tetrachlorodibenzofuran and (C) 2,3,4,5,6-pentachlorobiphenyl (from Fennell et al., 2004b).

dehalogenation in sediments is an important environmental process because it has the potential of decreasing the toxicity of organohalides such as PCDD/Fs. Dehalogenation may also be advantageous in designing contaminant treatment trains, since lesser chlorinated congeners are more susceptible to subsequent aerobic degradation.

4. PRIMING DEHALOGENATION

Recent research, including our own (Vargas et al., 2001; Fennell et al., 2004a; Ahn et al., 2005), has shown the potential for stimulation of dechlorination of halogenated organics in sediments under a variety of conditions. Biostimulation may be achieved through the addition of electron donors and/or of halogenated co-amendments that serve as more bioavailable electron acceptors.

Hydrogen is used by many dehalogenating bacteria and has also been shown to enhance dechlorination of PCDD residues in contaminated sediments (Albrecht et al., 1999). Hydrogen can be supplied directly or indirectly, at different biologically maintained levels, through the addition of fermentable organic compounds that serve as hydrogen donors (Fennell et al., 1997; Fennell and Gossett 2003; Yang and McCarty, 1998). In strict competition for hydrogen, dehalogenating bacteria can out-compete sulfate reducers. For example, the hydrogen threshold achieved by tetrachloroethene-dechlorinating populations in estuarine sediments was 0.5 nmol, less than that of the hydrogen level achieved by sulfate reduction in the same system (Mazur and Jones, 2001). Löffler et al., (1999) provided clear evidence for the role of hydrogen threshold concentrations in determination of electron accepting processes, also showed that thermodynamic constraints, i.e., uptake and energy yield, favor utilization by dehalorespirers in the presence of organohalide substrates. Clearly, all of these factors are important to consider when attempting to stimulate dehalogenation *in situ*.

The addition of alternate halogenated compounds, referred to as "priming" agents, has previously been shown to enhance dechlorination of PCBs and PCDDs (Beurskens et al., 1995; DeWeerd and Bedard 1999; Albrecht et al., 1999; Cho et al., 2002). For example, brominated compounds stimulated the dechlorination of PCBs in freshwater sediments (Bedard et al., 1998; DeWeerd and Bedard, 1999), monobromo-dibenzo-*p*-dioxin stimulated dechlorination of historically contaminated PCDDs (Albrecht et al., 1999), and bromophenols stimulated dechlorination of PCDDs in estuarine sediments (Vargas et al., 2001). This approach has been used with some success in small scale field studies (Bedard, 2003).

We previously demonstrated that dechlorination of 1,2,3,4-TeCDD was more extensive in heavily contaminated estuarine sediments from the Arthur Kill, an estuarine inlet located between Staten Island, New York and New Jersey, USA, when pre-enrichment on bromophenols was performed (Vargas et al., 2001). Bromophenol also stimulated 1,2,3,4-TeCDD dechlorination in sediments from San Diego Bay under methanogenic and sulfidogenic conditions (Ahn et al., 2005; Figure 3). We hypothesized that co-amendment of halogenated compounds with greater structural similarity to PCDD/Fs would be more effective at stimulating dechlorination of PCDD/Fs than simpler halogenated substrates such as bromophenols. Also, PCDD dechlorination should be more easily stimulated in sediment previously contaminated with other chlorinated compounds than in pristine sediments. Further investigation demonstrated that the most effective stimulation of 1,2,3,4-TeCDD and 1,2,3,4-TeCDF dechlorination occurred with co-amendment of 25 μM of 1,2,3,4-tetrachlorobenzene (TeCB), 2,3,4,5-tetrachloroanisole (TeCA), 2,3,4,5-tetrachlorophenol or 2',3',4'-trichloroacetophenone as substrate analogs plus 500 μM lactate and 500 μM propionate as electron donors (Ahn et al., 2005; Figure 5).

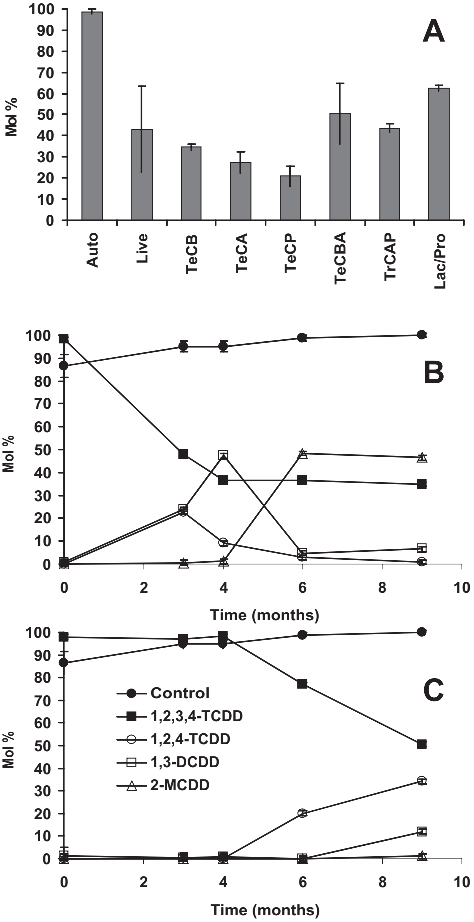

Figure 5. 1,2,3,4-TeCDD dechlorination in Paleta Creek sediment slurries under different electron donor and halogenated co-amendment conditions over 10 months with a 3-month re-amendment interval (A) Mole percent of 1,2,3,4-TeCDD remaining after 15 months; (B) time course of 1,2,3,4-TeCDD dechlorination in TeCB plus lactate/propionate-amended slurries and (C) time course of 1,2,3,4-TeCDD dechlorination in TeCA plus lactate/propionate-amended slurries (Ahn et al., 2005).

Similarly, dechlorination of 1,2,3,4-TeCDF to tri-chlorinated and di-chlorinated daughter products was significantly enhanced by TeCB and TeCA. These results suggest that halogenated aromatic compounds with structural similarity to 1,2,3,4-TeCDD/F stimulate bacteria with the ability to dechlorinate chlorinated dibenzo-p-dioxin and furans. Of critical importance is that these amendments stimulate the desirable lateral dechlorination of PCDD/Fs (removal of chlorines at position 2 and 3) that ultimately results in detoxification. This study coupled with findings that some species of dehalogenating bacteria have multiple dehalogenases and dechlorination capabilities suggests that halogenated compounds other than CDD/Fs could be used to enrich and isolate organisms that have activity on CDD/Fs. Identification of naturally-occurring or less toxic halogenated co-amendments that are completely degraded in the sediment environment is desirable for field application.

5. DEHALOGENATION IN THE RHIZOSPHERE OF SALTMARSH PLANTS

An additional variable affecting contaminant degradation in estuarine ecosystems is the effect of wetland plants. Microbial activity is stimulated in the rhizosphere of plants and wetlands may play an important function in remediation of contaminated sites, potentially reducing the impact of contaminants. We have examined the effect of two salt marsh macrophyte species on the sediment microbial community responsible for the biotransformation of the flame retardant tetrabromobisphenol A (TBBPA) (Ravit et al., 2005). Sediments vegetated by two grasses, *Spartina alterniflora* and *Phragmites australis*, commonly found in eastern U.S. brackish marshes differed significantly in their capacity to biotransform the halogenated contaminant TBBPA. Differences in the rates of TBBPA dehalogenation suggests that there are functional differences in sediment microbial communities in vegetated versus unvegetated habitats, as well as differences in the microbial communities associated with *Spartina* and *Phragmites* rhizosphere sediments.

The final product of microbial reductive dehalogenation of TBBPA (Voordeckers et al., 2002; Figure 6) is bisphenol A (4,4'-isopropylidenediphenol), a known endocrine disrupter. We have not observed further anaerobic degradation of bisphenol A (Voordeckers et al., 2002). Microbial community diversity as measured by in situ sediment phospholipid fatty acid (PLFA) composition prior to TBBPA exposure, was found to be higher in the uncontaminated sediments; differences in microbial PLFA diversity were not seen in contaminated sediments associated with either the different plant species or unvegetated sediment. Understanding the effect estuarine vegetation has on dehalogenating microbial populations is necessary to predict the fate of TBBPA, as well as the production and accumulation of bisphenol in estuarine sediments

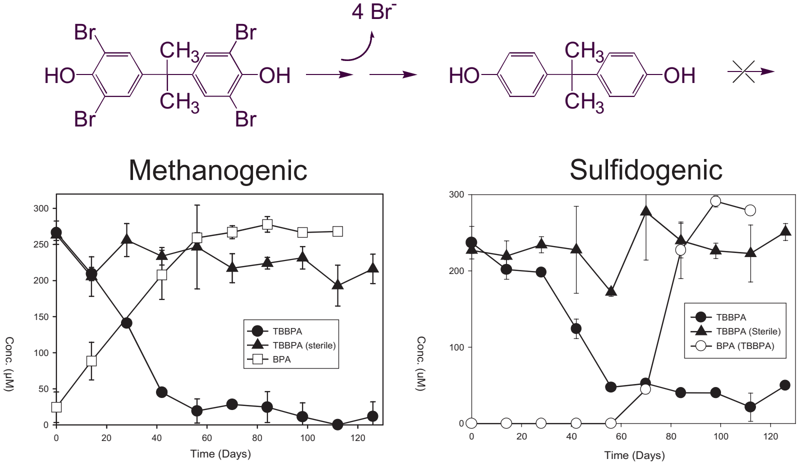

Figure 6. Dehalogenation of tetrabromobisphenol A in estuarine microcosms under methanogenic and sulfidogenic conditions (from Voordeckers et al., 2002).

6. CONCLUSIONS

Reductive dehalogenation is a promising mechanism for the removal of toxic organohalides from estuarine and marine sediments. Anaerobic dehalogenation of halogenated aromatic contaminants, such as PCDD/Fs and tetrabromobipshenol A, was readily promoted in estuarine and marine sediments from several sites. Co-amendment with more soluble halogenated compounds as "priming" agents greatly speeds PCDD/F dechlorination in anaerobic sediments. This stimulation was brought about in the presence of added electron donors lactate and propionate ensuring reduced conditions and adequate reducing equivalents to stimulate dechlorination. In highly organic sediments with adequate reducing power, it is possible that these halogenated additives may be just as effective without additional electron donors. While the process has much potential, a key aspect of the technology – the nature and capability of the intrinsic microbial community – is often unknown. The use of molecular tools for monitoring microbial activity coupled with the understanding of how redox processes affect dehalogenation may be used as a rapid screening method for determining whether a site is a good candidate for bioremediation and to tailor a bioremediation strategy for that site.

Sediment treatment is currently limited primarily to dredging with *ex situ* treatment or sequestration. New methods are needed for *in situ* containment and degradation of contaminants. Contaminated sediments often contain complex mixtures of pollutants, including PCBs, PCDD/Fs, chlorinated pesticides, halogenated flame retardants, polycyclic aromatic hydrocarbons (PAHs) and various heavy metals and the challenge of remediating the sites is significant. Our fundamental microbial studies are revealing the potential for remedial applications in aquatic systems and providing a foundation for development of integrated microbial and engineered approaches to enhance *in situ* bioremediation. Anaerobic reductive dehalogenation offers the most promising approach towards eventual detoxification and complete degradation of halogenated contaminant mixtures. *In situ* bioremediation combined with in-place containment through capping could avoid the problematic redistribution of contaminants that is associated with dredging and, where feasible, offer a more cost effective treatment alternative to dredging. Developing amendment technologies for enhanced microbial dehalogenation and understanding how amendment placement and mixing stimulates dehalogenation and impacts the fate and transport of organohalide mixtures is thus a high priority for the successful management of contaminated sediments.

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MOLECULAR TOOLS FOR MICROBIAL REMEDIATION - CONTAMINANTS UPTAKE, METABOLISM AND BIOSENSING

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Abstract: Microbial remediation has been used mainly for treatment of two types of pollutants - organic compounds (such as hydrocarbons originating from petroleum or solvents originating from various industries) and heavy metals. Molecular tools serve to improve the microbial processes in several ways – they contribute to optimization of the processes by genetic manipulations of the microorganisms, they improve the yield of bacterial products used for bioremediation (such as emulsifiers). In addition, molecular tools provide the means for constructing biosensors for monitoring the bioremediation processes.

Key words: bioremediation; oil degradation; biosensors; heavy metals; genetic engineering

1. INTRODUCTION

Molecular tools have been used in many areas of biotechnology and today it is inconceivable to think of science without these tools. The use of molecular tools for environmental purposes is also increasing, although more slowly than in other areas. Here I will discuss the use of molecular tools for treating pollution with heavy metals or organic compounds, mainly hydrocarbons. Molecular tools are useful for improving processes or reducing their costs. In addition, industrially-derived pollutants are often not degradable by natural bacteria, and their efficient removal requires genetic

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manipulations. Another area where molecular tools have been proved very useful is in constructing whole cell biosensors.

Bioremediation is a promising technology for treatment of environmental pollution. It has proved useful in treating oil pollution, as most of the components of crude oils are biodegradable, and are totally mineralized. Most of the bioremediation processes involve microorganisms, which are efficient in mineralizing hydrocarbons to water and carbon dioxide (Kirchmann and Ewnetu, 1998; Prince, 1993; Ron, 2000; Rosenberg et al., 1992; Rosenberg and Ron, 1996; Swannell et al., 1996). Bacteria are also potentially important for treatment of heavy metal pollution (Ron et al., 1992; Beveridge, 1997; Silver, 1994; Summers and Silver, 1978). They have been shown to bind heavy metals and remove them from the environment and in the case of mercury and chromium bacteria have been shown to change the valency of the metal, thus reducing its toxicity (Cervantes, 1991; Cervantes et al., 2001; Summers and Silver, 1978)

Bioremediation can often take place by the indigenous microorganisms that exist at the polluted site. However, these processes may take a very long time and some processes can not be carried out by the indigenous population. Therefore, molecular tools are used to set up efficient degradation processes that are well controlled. Genetic optimization of the bioremediation processes is required in a variety of cases. For example, while the lower molecular weight hydrocarbons are easily degradable, petroleum also contains a large fraction of hydrocarbons with low availability (such as PAHs). These hydrocarbons have a low bioavailability and are also difficult to degrade. Using molecular tools makes it possible to improve this process in several ways –

- Construct bacteria that produce surfactants to increase the bioavailability of the PAHs
- Construct bacteria that are more efficient in degrading the PAHs.
- Construct bacteria that function well in extreme conditions (cold or very hot areas, high salinity, or in sites when oxygen is limiting).

The use of molecular tools is even more important in the degradation of industry-derived hydrocarbons. For example, poly-halogenated hydrocarbons (PCB = polychlorinated biphenyl) are not natural compounds and bacteria that can degrade them are not common. Using molecular tools it was possible to construct bacteria that are capable of degrading halogenated hydrocarbons (Kumamaru et al., 1998; Suyama et al., 1996), under the desired set of conditions.

2. MICROBIAL BIOREMEDIATION

2.1 General Strategies for Improving Bioremediation of Organic Compounds

In principle, the following molecular tools are designed to improve bioremediation of organic compounds –

1. Construction of bacteria capable of utilizing multiple types of compounds.

Bacteria are specialists and contain a limited set of enzymes. Thus, bacteria that utilize aliphatic hydrocarbons will often not utilize aromatic hydrocarbons, phenols etc (Ron, 2000; Rosenberg and Ron, 1996). In order to overcome this problem, it is possible to introduce genes from a bacterium with one set of capabilities, into another bacterium with a complementary set. As many of the genes coding for degradation of aromatic hydrocarbons are found on plasmids (Ashok et al., 1995; Assinder and Williams, 1990; Chakrabarty et al., 1973; Chakrabarty, 1992; Dunn and Gunsalus, 1973; Franklin et al., 1981; Frantz and Chakrabarty, 1986; Furukawa and Chakrabarty, 1982; Sanseverino et al., 1993; Shields et al., 1995) it is quite easy to transfer these plasmids into bacteria that encode degradation of aliphatic hydrocarbons (Nusslein et al., 1992; Van Beilen et al., 1994).

2. Construction of more efficient bacteria

Improving the efficiency of certain processes can be achieved by manipulating the genes. It is possible to increase the copy number of desired genes, and it is also possible to increase their expression by changing the strength of the promoter, or the conditions under which it is activated.

3. Construction of hydrocarbon utilizers with better fitness

Often the polluted areas are in extreme environments, in which the growth of most bacteria is inhibited. However, bacteria can be selected that grow well in extreme temperatures (hot or cold) (Whyte et al., 1998), low oxygen tension, high salinity etc. These bacteria can be used as recipients for bioremediation-related genes, to produce bacteria with high fitness and the desired metabolic capabilities (Ciaramella et al., 2002; Colwell, 1997; Herbert, 1992; Hunter-Cevera, 1998; Margesin and Schinner, 2001; Mock and Thomas, 2005; Niehaus et al., 1999; van den Burg, 2003).

4. Selection of bacteria with new enzymatic capabilities

Many industrial pollutants are not degradable by natural bacteria. A good example is chlorinated organic compounds, which are among the most significant pollutants in the world. Using sophisticated, *in vitro* selection methods, it was possible to obtain bacteria that can degrade some of the "undegradable" compounds. The techniques used to achieve this included *in vitro* DNA shuffling and subunit or domain exchanges between dioxygenases of different bacterial origins. For example, sequential use of anaerobic halorespiring bacteria, key players in biological dehalogenation processes, and aerobic bacteria whose oxygenases are modified by directed evolution could lead to efficient and total degradation of highly chlorinated organic pollutants. This procedure yielded newly-evolved enzymes which acquired novel and enhanced degradation capabilities of xenobiotic compounds, such as polychlorinated biphenyls, trichloroethylene and a variety of aromatic compounds (Furukawa and Kimura, 1995; Furukawa, 2000, 2003).

5. Molecular tools in the production of emulsifiers

As already mentioned, emulsifiers improve the availability of polyaromatic hydrocarbons (PAHs) and other hydrocarbons with low water solubility (Barkay et al., 1999; Dagher et al., 1997; Desai and Banat, 1997; Marin et al., 1995; Ron and Rosenberg, 2001; Rosenberg and Ron, 1997; Rosenberg and Ron, 1999). Emulsifiers produced by bacteria are effective, non toxic and environmentally friendly. To use these emulsifiers it is possible to add bacteria that produce them, or – alternatively – it is possible to produce the emulsifiers by fermentation and add them to the process. Either way, genetic manipulations can be used which improve and increase the production of the emulsifiers (Bekerman et al., 2004; Navon-Venezia et al., 1995; Toren et al., 2001; Toren et al., 2002a; Toren et al., 2002b; Toren et al., 2002c).

2.2 Treatment of Heavy Metal Pollution

Bacteria can bind heavy metals, thus concentrating them into small volumes and removing them from the environment. In addition, bacteria can detoxify the heavy metal by chemical transformation involving reduction, oxidation, methylation and demethylation (Beveridge et al., 1997; Ron et al., 1992; Silver, 1994; Silver and Phung, 1996; Summers and Silver, 1978). One of the best understood biological systems for detoxifying organometallic or inorganic compounds involves the *mer* operon. Bacteria carrying this operon take up Hg^{2+} into the cell and delivered it to the NADPH-dependent flavoenzyme mercuric reductase, which catalyzes the two-electron reduction of Hg^{2+} to volatile, low-toxicity Hg^0.

Here, like in the case of hydrocarbons, molecular tools can be used to obtain an improved process, bacteria which are more fit, or bacteria with broad detoxifying capabilities (Silver and Walderhaug, 1992; Silver, 1994, 1998).

2.3 Future Directions – Bioremediation of Explosives

Pollution of a different type is produced in our terror-struck world. Thus, there is an increasing use of explosives of a new type improvised explosives that are home produced. Unfortunately, these products are becoming quite common and create a problem, especially in countries suffering from terrorism. These improvised explosives are very sensitive, and are therefore difficult to remove to disposal areas. Although there are chemical solutions for the immediate neutralization of improvised explosives, these involve highly toxic materials and are often quite dangerous for the civilian population. Clearly, microbial bioremediation is the ideal solution. Because these explosives are not natural compounds, bacteria capable of degrading them are rare. Such bacteria can be constructed by the use of molecular tools, and have already proved effective in lab scale.

2.4 Monitoring the Efficacy of Bioremediation

The efficacy of bioremediation is determined chemically, by measuring the change in total pollutant concentrations, usually complemented by chromatographic results (gas chromatography, or gas chromatography-mass spectrometry). Recently, there are attempts to introduce biosensors, especially microbial whole cell biosensors, to monitor the rate of elimination of the pollutants (Burlage and Kuo, 1994; Daunert et al., 2001; de Lorenzo et al., 1993; Kohler et al., 2000; Paitan et al., 2003; Simpson et al., 1998; Sticher et al., 1997). These biosensors are constructed by molecular tools and consist of promoters from genes that are induced by the presence of the pollutant of interest. These promoters are fused to reporter genes whose expression can be easily monitored. An example of such biosensors is the strain of *Escherichia coli* K-12 that carries the promoter of the *xylS* gene - induced by aromatic hydrocarbons - from *P. putida* that has been fused to a promoterless luciferase operon (from *Vibrio harveyi* or firefly). The expression of luciferase is measured by light emission. It is induced in the presence of aromatic compounds. The lower detection limit for *m*-xylene was 5 mM (Kobatake et al., 1995). Similar biosensors were constructed that can monitor the level of additional environmental pollutants, such as toluene or octane (Applegate et al., 1998; Sticher et al., 1997). Recently new whole cell biosensors have been constructed in which the monitoring of gene expression is electrochemical. These promoter-based, whole cell biosensors are suitable for on line and *in situ* monitoring of pollutants (Biran et al., 1999; Biran et al., 2000; Paitan et al., 2004; Scott et al., 1997).

One of the advantages of biosensors is that they discriminate between the total pollutants and the bioavailable pollutants, the latter being at least as important as the value for total hydrocarbons. Biosensors constitute a cost-effective, convenient solution to monitoring the progress of bioremediation treatments. It is expected that the use of biosensors will become widespread, especially if further developments improve the capabilities for monitoring online and on site.

Indeed, recent publications describe the development of a "nano" electrochemical biosensor, with eight channels, that uses disposable miniature electrodes and is connected to a hand-held computer (Popovtzer et al., 2005). Another novel bioremediation device involves the development of a live cell array biosensor. This biosensor contains a high density array of immobilizing bacterial cells on the face of an optical imaging fiber. The bacteria are embedded in an array of microwells, each of which accommodates a single bacterium. The bacteria have been genetically engineered to respond to specific analytes, and a combination of many such bacteria enables the measurement of many individual cell responses. This whole cell biosensor platform is very sensitive since each cell acts as an individual, independent sensor. Averaging responses from multiple identical sensors improves the signal to noise ratio by the square root of the number of sensors. The optical imaging fiber-based single bacterial cell array is a flexible and sensitive biosensor platform that can be used to monitor the expression of different reporter genes and accommodate a variety of sensing strains (Biran, et al., 2004).

3. CONCLUSIONS

With the growing need for efficient and fast bioremediation of environmental pollution, increased use of molecular tools is inevitable. For bioremediation in open systems the introduction of genetically modified organisms (GMOs) may be resented. However, GMOs are in increasing demand for use in confined systems for recalcitrant industrial effluents (PCBs etc). In addition, the development of biosensors relies on genetically manipulated organisms. The use of these systems is bound to increase rapidly, as they are the only available tool for monitoring pollution on line, *in situ* and in a cost-effective manner.

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ROLE OF MYCORRHIZAL FUNGI IN PHYTOREMEDIATION AND TOXICITY MONITORING OF HEAVY METAL RICH INDUSTRIAL WASTES IN SOUTHERN POLAND

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Abstract: The chapter summarizes research carried out within the last 15 years on the role of mycorrhizal fungi in phytoremediation of zinc wastes located in southern Poland. The impact of various technologies on tailing material toxicity was described and physico-chemical features of the wastes were characterized. Symbiotic fungi are an important component of soil microbiota. As shown in a range of studies, properly developed mutual symbiosis enhances the survival of plants in polluted areas by for instance improving nutrient acquisition and water relations, thus supporting the success of bioremediation. In addition, mycorrhizal fungi were found to play an important role in heavy metal detoxification and the establishment of vegetation in strongly polluted areas. Fungal strains isolated from old zinc wastes also decrease heavy metal uptake by plants growing on metal rich substrata, limiting the risk of increasing the levels of these elements in the food chain. The effectiveness of the bioremediation techniques depends on the appropriate selection of both the plant and the fungal partners. The experiments pointed out that plants conventionally introduced in such places disappear relatively soon, while those appearing during natural succession are better adapted to harsh conditions. Symbiotic partners selected on the basis of such research are often the best choice for future phytoremediation technologies. Moreover, mycorrhizas of different types are also helpful in tailing material toxicity monitoring.

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Key words: phytoremediation; phytostabilization; mycorrhizal fungi; heavy metal toxicity; industrial wastes; native plants.

1. INTRODUCTION

Contamination of soil and water seriously threatens the environment and human health. The problem is especially grave in areas influenced by zinc and lead industry. In the south-western part of Poland metal ores have been exploited since the 10th century, when the surface ore concretes were used. Ore mining started in the 12th century (Szuwarzynski, 2000). Metasomatic ores occurring in triassic dolomites in Silesia, including the areas of Bytom, Olkusz and Chrzanow are of economic importance (Strzyszcz, 2003). At first, only lead and silver were excavated, mostly using hands and simple tools, and the rock pieces containing the ore were selected visually. Much later, the excavated material was crushed and centrifuged in water within hydrocyclones and the ores were collected from the bottom. This technology was not very efficient and the wastes that were deposited in heaps contained double the amount of precious metals than the wastes produced with technologies developed later. On the other hand, however, the granularity of the material was 2–7 mm and the wastes were not so easily translocated by wind. Since 1970 the excavated ores have been subjected to flotation processes. In this case, the metals were collected from the top of the tanks. Waste material obtained as a by-product of flotation is deposited within the area of ore exploitation. The solid fraction is used to form tailing ponds filled with the liquid phase. Due to differences in the technologies of flotation, the wastes differ in metal content and potential toxicity.

Post-flotation wastes are an extremely harsh substratum, poorly suited for plant growth. The sediment is composed of grains, mostly between 0.1–0.02 mm in diameter, making it extremely difficult for any biological reclamation (Strzyszcz, 1980, 2003). The density of this material is higher than that found in natural soils, and the low porosity results in unfavourable air-water conditions, restricted water infiltration during rainfall and decreased water recharge by capillary rise from deeper layers during dry periods. These factors favour wind erosion in dry periods and water erosion during rainfalls (Strzyszcz, 1980, 2003). The slopes of the heaps are of similar material to that of sediment ponds, although the grain size is often larger. In addition, the slopes of the heaps slide down and are difficult to stabilize. Industrial wastes are comparatively easy to manage while the exploitation of the ores is still being carried out and water is pumped into the sedimentation tank. In the case of the Trzebionka waste, about 560 000 m^3 of water per year is being pumped inside the pond, more than the atmospheric precipitation estimated for this area. The hydrological situation could worsen significantly after exploitation stops in the years to come.

The chemical composition of the tailing material is also unfavourable, because the carbonate content is over 75%. High concentrations of Ca^{2+}, SO_4^{2-} and low Na^+, K^+, Mg^{2+}, Cl^- and HCO_3^- concentrations are also found in these tailings. The original tailing material contains practically no organic matter and is very low in P and N. Although the heavy metal levels exceed several times the threshold values for arable soils, due to the alkaline pH (7.4) their availability is low. Therefore the wastes are not severely phytotoxic, at least the material remains in place and is not moved by wind to areas of lower pH. The dust originating from the waste heap area often contains high levels of Zn (12 067 mg/kg), Pb (2 372 mg/kg) and Cd (108 mg/kg), which poses serious health hazards for plants and animals.

Additionally, certain ores are rich in Tl. This fact was unknown until 1995 when monitoring assays aimed at determining the Tl content in magpie feathers and rodent tissues were carried out (Dmowski, 2000). The reservoir in Bukowno was pointed out as a source of this element. Inhabitants of villages located close to the wastes are at high risk due to the consumption of vegetables containing Tl. Both the waste deposits and the surrounding areas need to be included in restoration/remediation programs. In this particular situation, technologies based on physico-chemical extraction methods enabling detoxification/deactivation and removal of toxic compounds from the soil are ruled out, as the waste area is too large and such technologies totally destroy soil microorganisms.

Another important aspect of zinc waste deposits is that the flora developed during spontaneous succession on the heavy-metal rich tailing material is very interesting. Such areas are rich in rare plants such as *Biscutella laevigata,* which is found in Poland only in two localities (Orlowska et al., 2002). Actually, the whole communities are worth protecting. Conventional restoration methods, such as reforestation, would destroy this kind of vegetation. When protected, this site could be converted as it is in order to establish a refuge for rare and endangered plant species (Bizoux et al., 2004). The use of native plants for the revegetation of damaged areas is presently believed to be the best choice (Urbanska, 1995). Intensive studies on the mechanisms allowing plants to survive under such extreme conditions are of utmost importance. The case of the about 100-year-old calamine waste in Boleslaw near Olkusz is the best example of intensive studies of such areas (e.g. Grodzinska et al., 2001; Wierzbicka and

Potocka, 2002; Szarek-Lukaszewska and Niklinska, 2002; Pielichowska and Wierzbicka, 2004; Biskup and Izmailow, 2004).

Plant-based technologies in industrial areas are mostly limited to phytostabilization, involving the use of plants to stabilize the contaminated tailing material, in order to minimize leaching and wind or water erosion (Pierzynski et al., 2000, 2002). In such cases, the tailing material should be managed to minimize the uptake of heavy metals by plants. Another technology termed phytoextraction might be useful if areas surrounding the industrial wastes become strongly polluted by dust particles from the wastes. They contain less heavy metals, although possibly of higher availability. In such cases, the aim would be to maximize the amount of metals in the soil solution that are available for uptake by plants. Phytoremediation is the only possible alternative; it involves the use of plants in the process of decreasing the level of toxic compounds in soil, in stabilizing the soil and inhibiting erosion. Recently it was demonstrated that phytoremediation can be enhanced by the use of appropriately selected microorganisms, such as mycorrhizal fungi (Hildebrandt et al., 1999). The investigations on such fungi occurring on zinc and lead ore wastes have been carried out in southern Poland for the last 15 years. The aim of this chapter is to summarize the state of knowledge.

2. RESTORATION TECHNIQUES IN POLAND

Until flotation processes were introduced, zinc ore wastes were simply abandoned and spontaneous vegetation usually developed within 20–30 years. In the region of Olkusz, forests of the *Tilio-Carpinetum* type dominated by *Fagus sylvatica* developed on areas previously used for surface exploitation of the ores. Still, many depressions termed “warpie” exist, being a visible trace of the digging for ores. Primarily, the wastes were composed of particles of about 3–7 mm in diameter and containing 2% of the ore. The wastes of the “Matylda” mine in Chrzanow can serve as an example. Although the risk of wind erosion is comparatively low there, the toxicity of this waste is higher than of the later produced wastes. Forty years of spontaneous succession resulted in the creation of vegetation dominated by *Pinus silvestris* and *Betula verrucosa* accompanied by *Dianthus carthusianorum*, *Trifolium pratense*, *Silene vulgaris*, *Equisetum pratense*, *Phragmites australis*, *Epipactis helleborine, E. atrorubens* and *Dactylorhiza majalis*. Most waste deposits from this period disappeared, as the material was removed for road construction to fill the space below the asphalt layer. With time, the efficiency of metal extraction of the flotation technology was enhanced and the content of metals left in waste material was reduced to ca. 1%. At the same time, however, the diameter of the particles deposited decreased to 0.1–0.03 mm, which seriously increased the risk of wind erosion.

An adopted remediation practice consists of covering the waste pile with a layer of humus or other material to prevent erosion. The use of clean soil is too costly for large areas. The thickness of the "organic" material layer varies from 20 cm to 2 m, depending on location. Such a layer allows for easier establishment of vegetation, by providing a barrier for the factors limiting plant growth and limits the transfer of heavy metals into the plant material. According to Strzyszcz (2003), the metal content in fresh/dry leaves of poplar grown on this type of wastes may reach over 100 mg/kg of Pb and over 2000 mg/kg of Zn. Mixed grass species introduced into bare wastes may contain even 454 mg/kg of Pb, almost 700 mg/kg of Zn and 20 mg/kg of Cd.

The most commonly introduced tree species include birch, pine and poplar. Shrubs such as *Hippopheae rhamnoides*, *Eleagnus angustifolius*, *Robinia pseudoacacia* and *Physocarpus opulifolius* also establish comparatively well. The most often used grass species include *Festuca ovina*, *F. rubra*, *Phleum pratense*, *Poa pratensis*, *Lolium perenne*, *Dactylis glomerata*, *Arrhenatherum elatius*, *Bromus inermis* and *Festuca pratensis*. The grasses are often supplemented with *Medicago sativa* and *Trifolium pratense*. According to long-term observations carried out on zinc wastes in Trzebionka near Chrzanow, the introduced plant populations dramatically decrease with time, and almost none of the species are left on the oldest part of the waste. Much more stable vegetation communities arise from plants that appear on the wastes spontaneously, but their establishment and the creation of stable communities takes a long time.

Spontaneous succession on zinc wastes leads towards the development of interesting vegetation that often resembles that of the 100-year-old calamine area remained after surface exploitation of ores, and is similar to the newly developed industrial heap of the Boleslaw plant. The non-restored part is inhabited by very interesting vegetation including rare species. The only species restricted to soils rich in heavy metals is *Armeria maritima* subsp. *halleri* (Szafer, 1959). It is a mycorrhizal species, although the level of colonization is rather low (Pawlowska et al., 1996). Also *Biscutella laevigata*, *Silene vulgaris*, *Gypsophila fastigiata*, *Cerastium arvense* (Grodzinska et al., 2001) can be found there.

3. THE IMPORTANCE OF MYCORRHIZAL FUNGI IN RESTORATION OF INDUSTRIAL WASTES

Plants need appropriate below-ground ecosystems, especially at difficult sites. Mycorrhizal fungi enhance root absorption area up to 47-fold (Smith and Read, 1997). The fungi provide nutrients and water otherwise not accessible for plants (Cui and Nobel, 1992; George et al., 1992; Nadian et al., 1997) and facilitate the establishment and survival of vegetation under stress conditions (Jasper et al., 1989; Smith et al., 1998). The fungi also stabilize the tailing material with the net of hyphae and improve its structure, as they produce substances that bind soil particles, leading to the formation of soil aggregates (Thomas et al., 1993, Wright and Upadhyaya, 1996, Jastrow et al., 1998). The compounds produced by the extraradical mycelium can also take part in heavy metal chelation. Fungi are known to be able to accumulate significant amounts of heavy metals (Gadd, 1993) varying from a few percent to 20% of dry mass (Tobin et al., 1984), suggesting that microbial biomass may affect the mobility of metals in the soil system. According to the calculations by Söderström (1979), the surface of interaction between fungi and soil is up to 0.14 m^2 in 1 g of soil. They can remove metals from the wastes both by metabolism dependent (bioaccumulation) or independent (biosorption) processes (Gadd, 1993). In the second case, both live and dead biomass can be involved (Volesky and Holan, 1995). The components of the fungal cell wall can be very efficient in binding heavy metals due to the presence of free amino, hydroxyl, carboxyl and other groups (Gadd, 1993). Therefore, saprobic fungi can be commercially grown in bulk culture and their either live or dead biomass used as biosorbents for heavy metals (Morley and Gadd, 1995; Kapoor and Virarghavan, 1995; Mullen et al., 1992; Fomina et al., 2005). Similar phenomena occur in ectomycorrhizal (Denny and Wilkins, 1987; Turnau and Dexheimer, 1995), ericoid (Bradley et al., 1982) and arbuscular mycorrhizal fungi (Joner et al., 2000; Gonzales-Chavez et al., 2002, 2004). Some of these microorganisms can also precipitate heavy metals outside the mycelium e.g. by producing various organic acids or enzymes such as the acid phosphatase (Turnau and Dexheimer, 1995) or pigments, which additionally prevents the migration of metals.

Arbuscular mycorrhiza (AM), occurring in 80% of plant species and formed by about 120 species of fungi belonging to the Glomeromycota (Schüßler et al., 2001), is the most widespread type of symbiosis between fungi and plants. Besides the formation of the extraradical myceliar net that intensively penetrates the substratum (feature common to all types of mycorrhizas), it forms intraradical hyphae that penetrate intercellular spaces and enter cortical root cells. The formation of comparatively short-lived structures called arbuscules is crucial for the functioning of the whole symbiosis (Smith and Read, 1997). This is the place where the arbuscular cell wall is lined by plant plasmalemma, and the exchange of substances takes place. AMF have not been shown to produce organic acids such as oxalic acid, however, glomalin, a protein produced by these fungi, seems to be efficient in sequestering Cu, Cd, Pb and Mn (Gonzales-Chavez et al., 2004). According to Joner and Leyval (1997), the efficiency of protection depends on the AMF isolate. These authors have also shown that no inhibition of mycelium growth was observed even at 20 mg of $NH_4NO_3^-$ extractable Cd/kg of substratum.

Retention of heavy metals in extraradical mycelium of ectomycorrhizas was first proposed as a tolerance mechanism by Denny and Wilkins (1987). The fungi can detoxify metals by intracellular processes (Jentschke and Godbold, 2000; Blaudez et al., 2000). A variety of membrane transporters controlling the trafficking of metal ions have been identified recently in plants and microorganisms (Clemens, 2001). Intracellular detoxification in fungi and plants is attributed to metal chelation by cysteine-rich peptides such as reduced glutathione, phytochelatins and metallothioneins (MeT) (Cobbett and Goldsbrough, 2002; Blaudez et al., 2000). Cd-MeT was shown to take part in detoxification of heavy metals in the ectomycorrhizal fungus, *Paxillus involutus* (Courbot et al., 2004), while Cu^{2+}-MeT was extracted from *Laccaria laccata* and *P. involutus* and their production was correlated with the tolerance to copper (Howe et al., 1997). Despite the increase of glutathione production, the content of phytochelatin was not increased, suggesting that at least in this fungus the cadmium detoxification mechanism is different from the mechanisms observed in the host plant. MeT-like sequences were identified in the ectomycorrhizal fungus *Pisolithus tinctorius* (Voiblet et al., 2001) and in the arbuscular fungus *Gigaspora rosea* (Stommel et al., 2001) although the metal sequestration capacity and actual MeT-like nature was not determined until recently. The identification and functional characterization of an MeT-encoding gene from *Gigaspora margarita* was demonstrated by Lanfranco et al. (2002), and in addition the differences in gene expression in symbiotic and pre-symbiotic stages were shown.

Mycorrhizal fungi qualitatively and quantitatively influence the microbial population of the mycorrhizosphere. They are usually accompanied by bacteria such as legume symbiotic nodular bacteria, plant growth promoting rhizobacteria (PGPR), mycorrhiza helper bacteria (MHB) and saprobic fungi. As these organisms influence plants either by interactions with abiotic (Turnau and Kottke, 2005) and biotic components of the soil (Azcón-Aguilar and Barea, 1996), or by stimulating plant growth through the production of vitamins and hormones (Barea, 1997, 2000), they should be included in the optimization of the restoration processes as well as mycorrhizal fungi.

3.1 Role of Arbuscular Mycorrhizal Fungi in Revegetation of Zinc Wastes in Poland

Arbuscular mycorrhiza has been investigated on industrial wastes rich in heavy metals in southern Poland since 1990. The appearance of arbuscular mycorrhizal fungi at the zinc wastes usually takes several years. The number of spores in the substrata of different age varies from 0 to 100 spores per 100 g in revegetated areas The restoration practices, including scarification and planting of trees or covering the original tailing material with material result at least in doubling the number of spores and percentage of alive spores (Zubek et al., 2003). The tailing material deposited in the heaps and the liquid waste pumped into the sedimentation tanks is devoid of arbuscular mycorrhizal fungi. The humus that is used to cover the heaps unfortunately usually does not contain fungal propagules. In addition, the use of inorganic fertilizers can slow down the colonization of the wastes by arbuscular mycorrhizal fungi (AMF).

Commercial cultivars used to restore waste areas are often selected on the basis of the highest biomass production at high fertilizer input. That often correlates with low or even no dependency on mycorrhizal fungi. After ca. 30 years these plants are substituted by spontaneous vegetation, which develops slowly but is able to build stable communities. Such succession of plants was documented at Trzebionka zinc wastes, where the highest parts of the heap are the youngest, and parts at the basis are the oldest (unpublished data). The first plants to appear on bare wastes were nonmycorrhizal members of Caryophyllaceae (*Cerastium* spp., *Silene vulgaris*) and Brassicaceae (*Cardaminopsis arenosa*). They are, however, ineffective in covering the tailing material. These plants are followed by the facultatively mycorrhizal ones, establishing better if mycorrhizal fungi are present. Among them, grasses such as *Molinia caerulea*, *Agrostis gigantea*, *Bromus inermis*, *Calamagrostis epigejos*, *Corynephorus canescens*, *Dactylis glomerata*, *Festuca tenuifolia* and *F. trachyphylla* are the most expansive species, being very promising candidates for the phytostabilisation of zinc wastes. Grasses collected from zinc wastes are usually very strongly mycorrhizal. In some cases (e.g. *Festuca tenuifolia*), mycorrhizal colonization and arbuscular richness were 2–5 times higher than in natural stands (outside the industrial area). Among cultivars of grasses that were possible to be studied under laboratory conditions, two cultivars of *Festuca* *rubra* and one cultivar of *Lolium perenne* showed increased growth and one showed decreased growth in response to mycorrhiza (*Poa pratensis* cv Alicja). The positive reaction to mycorrhiza may be, however, visible in the second year of cultivation, e.g. in improved survival of new seedlings (Jeffries et al., 2003). Although the mycorrhizal colonization of grasses on zinc wastes was usually high, there were some exceptions. The parameters of mycorrhization (mycorrhizal colonization, m% and arbuscule richness, a%) were much lower in the case of the same plants occurring in places where the fresh liquid waste was spilled from damaged pipes, which clearly demonstrates that the waste is more harmful at the beginning. The main stress is due to the lack of water holding capacity and low nutrient pool. The importance of proper nutrition is well visible at the zinc mound of Boleslaw, where a limited area was covered with material of similar toxicity, but a much higher N and P content and better water sorption capacity. This resulted in improved development of plants and higher viability of AM spores as shown by INT reaction (Zubek et al., 2003).

Taking into account the vegetation on all investigated zinc wastes, it is interesting to note that such sites are inhabited by many plants originating from wet areas (*Molinia caerulea*, *Carex flacca*, *Sanguisorba officinalis*, *Valeriana officinalis*, *Phragmites australis*). They are often resistant to heavy metals due to the increased content of Si in their tissues (Kabata-Pendias and Pendias, 1999). They easily survive periodical changes of water level and develop mycorrhiza when water levels are low. At the same time, there are also plants typical for xerothermic grasslands (e.g. *Poa compressa*, *Scabiosa ochroleuca*, *Sedum acre*, *Dianthus carthusianorum*, *Linum catharcticum* and *Thymus serpyllum*), that are often adapted to dry conditions and to high content of Ca in the soil.

The diversity of AMF was studied in the calamine area in Boleslaw and on the zinc wastes generated by the ZG Trzebionka. Morphological analysis of spores from the 100-year-old calamines revealed the presence of *Glomus aggregatum*, *G. constrictum*, *G. fasciculatum*, *G. pansihalos* and *Entrophospora* sp. (Pawlowska et al., 1996). Nested polymerase chain reaction (PCR) with taxon-specific primers was used to identify the species *G. moseae*, *G. intraradices*, *G. claroideum*, *G. gerdemannii* and *Paraglomus occultum* (Turnau et al., 2001). Additional morphological analysis added *G. fasciculatum* and *G. aggregatum* to the list of AMF species of zinc wastes of ZG Trzebionka (unpublished results). These studies were followed by the localization of heavy metals using rhodizonate salts (Turnau, 1998; Turnau et al., 2001). It was shown that fungi isolated from industrial wastes decrease the metal uptake into the roots and shoots of *Plantago lanceolata*, when compared to strains originating from soils of low heavy metal content

(Orlowska et al., 2005). In addition, in spores of *Glomus intraradices* isolated from the zinc wastes of ZG Trzebionka, characteristic depositions were observed within the periplasmic space, between the inner layer of the wall and the plazmolemma. The accumulation of heavy metals within these depositions was confirmed by EDS analysis. The method was also used to estimate the percentage of the mycelia showing increased metal accumulation. In the case of *Euphorbia cyparissias* (Turnau, 1998) about 80% of the intraradical mycelium showed increased content of heavy metals. On the contrary, roots of *Fragaria vesca* from the same industrial wastes that were selected for molecular studies on fungal identity showed increased metal accumulation only in the case of *G. mosseae,* which is one of the least efficient colonizers (below 10%). Under field conditions the plants are inhabited by several different strains or species. For practical reasons, the research under laboratory conditions often has to be carried out using single strains. It is known that the response of the plant can vary depending on the AMF isolate and species (Streitwolf-Engel et al.; 1997, van der Heijden et al., 1998). AMF were already reported to decrease or increase heavy metal uptake by plants (Leyval et al., 1997; Khan et al., 2000). They can provide a potential for decreasing health hazards of edible plant production and improve sustainable agriculture and phytoremediation technologies, including phytoextraction (Jurkiewicz et al., 2004; Turnau and Mesjasz-Przybylowicz, 2003). Research on heavy metal uptake by plants growing on zinc industrial wastes treated with EDTA using 15 cultivars of *Zea mays* and *P. lanceolata* demonstrated that EDTA strongly decreased the activity of the fungal alkaline phosphatase (indicator of fungal viability); still, the treatment did not totally eliminate the arbuscular mycorrhizal fungi (AMF) from the soil. A modification of the appearance of AMF structures within roots of plants cultivated in EDTA-treated soil was observed. Among the heavy metals studied, the highest impact of EDTA treatment on heavy metal uptake into shoots was noted in the case of Pb. EDTA significantly increased the Pb level in shoots of mycorrhizal plants, while compared with mycorrhizal samples not subjected to EDTA. Between samples treated with EDTA, mycorrhizal plants of 6 cultivars showed a higher Pb content in shoots than nonmycorrhizal ones. Significant differences in heavy metal content in plant material were demonstrated between the varieties tested.

Plantago lanceolata, a plant highly responsive to mycorrhiza, was used to estimate the differences in Cd, Zn and Pb uptake by plants inoculated with different AMF strains (Orlowska et al., 2005), showing that heavy metal uptake clearly depended on the fungal strain used. Plants inoculated with AMF strains originating from soils not affected by heavy metals had higher metal concentrations in tissues than plants inoculated with strains from polluted areas. Plants growing on zinc wastes often contain high metal levels, which can be transferred into the food chain. The introduction of efficient heavy metal accumulating mycorrhizal fungal strains can decrease the uptake of metals into the shoots, while the available pool of metals could be stored within the root system.

Still, the practical application of AMF awaits further investigations. So far, several weak points of the technology have been demonstrated, concerning e.g. the proper selection of plant species (Pierzynski et al., 2002). Restoration can be further enhanced by additional inoculation with suitable symbiotic and saprophytic rhizosphere microorganisms isolated from heavy metal-polluted soils (Turnau et al., 2005).

3.2 Ectomycorrhizal Fungi in Zinc Waste Restoration

Zinc wastes in Poland are often spontaneously inhabited by pines, birches, willows and poplars. Pines (*Pinus sylvestris* and *P. nigra*) and birches are also introduced in such areas on a wide scale. The growth of these trees is slow and in many cases the herb layer is poorly developed; therefore, the role of the trees in limiting wind erosion is not equivalent to the grass cover. The trees are also able to establish on calamine areas that remained after surface exploitation of the ore, although their growth is much slower. Macrofungi occurring on zinc wastes in southern Poland were investigated. So far, almost 70 species were recorded, 40–70% of which are ectomycorrhizal (Mleczko, 2004), and forming a fungal mantle on the surface of the root and the so-called Hartig net (mycelium penetrating between cortical cells of the root). Most mycorrhizal fungi occurring on wastes have a broad range of hosts. Fungi forming symbiosis exclusively with pine include *Chroogomphus rutilus*, *Suillus luteus* and *Rhizopogon roseolus,* and are very common in all investigated localities. There are also species associating exclusively with birch, such as *Lactarius pubescens*, *L. torminosus* and *Leccinum scabrum*. The above mentioned species, especially those that accompany pines, formed abundant fruitbodies on zinc wastes, sometimes more abundant than mycorrhizas observed in the soil samples collected from the same wastes. Out of the 33 000 short roots analysed, only 20% were found to contain live mycorrhizas and about 43% to be nonmycorrhizal short roots (Turnau et al., 2002). Molecular and classical analysis of the mycorrhizas showed that *S. luteus* and *R. roseolus* represented up to 7 and 3% respectively, despite abundance of their fruitbodies. The most frequent was an undidentified *Pinirhiza arenosa* forming ca 70% of all mycorrhizas followed by *Tricholoma scalpturatum*, *Hebeloma mesophaeum* and mycorrhizas formed by members of

Thelephoraceae (Mleczko, 2004). Protective properties of ectomycorrhizal fungi depend on the species of the symbiotic fungus, the water absorbing capacity of the mantle, the production of pigments and organic acids by mycelium building the mantle and also forming extraradical mycelium that penetrates the soil. Research on differences between mycorrhizas from Polish wastes was carried out using electron energy loss spectroscopy connected to a transmission electron microscope (EELS/TEM) (Turnau et al., 1996), energy dispersion spectroscopy (EDS) connected to a scanning electron microscope (Turnau et al., 2002) and particle-induced X-ray emission (PIXE) (Turnau et al., 2001). The material was prepared in various ways, to avoid redistribution of elements during the preparation (Turnau and Kottke, 2005). The data show that *S. luteus* and *R. roseolus*, both belonging to the suilloid type, were the most efficient in filtering; unfortunately, they form few mycorrhizas. The practical application of ectomycorrhizas seems to be more complicated than the introduction of AMF that have almost no competitors in this tailing material (at least at the beginning of the succession). While AMF fungi slowly colonize the tailing material ectomycorrhizal spores are more easily transported by wind and we have no possibility to stimulate the success of the most effective mycorrhizal communities – they can be out competed by other noneffective ectomycorrhizal species. The ideal fungus has to be adapted to the site characteristics, be aggressive enough to spread within the root system and be effective in plant protection. Additionally, it has to multiply well *in vitro* and its tolerance to heavy metals has to be demonstrated, as not all strains isolated from the polluted site are equally tolerant to heavy metals (Blaudez et al., 2000).

4. USE OF MYCORRHIZAL FUNGI AS INDICATORS OF ZINC WASTE RESTORATION

The use of mycorrhizal parameters as an indicator of changes occurring during soil restoration and a tool for biomonitoring the soil quality has already been addressed by Lovera and Cuenca (1996) and Haselwandter (1997). Diverse mycorrhizal fungi can be used, including ectomycorrhizal (fruitbodies and mycorrhizas) and arbuscular species (Leyval et al., 2002; Orlowska et al., 2002). The changes occuring on zinc wastes due to e.g. introduction of trees could be easily evaluated using mycorrhizal parameters obtained for *P. lanceolata*. The highest differences between the restored and the non-restored part of Boleslaw waste were found in relative mycorrhizal colonisation and relative arbuscular formation. The mycorrhizas from calamine wastes differed from the material collected from the reference area in relative arbuscule richness, but not in mycorrhizal colonization intensity (Orlowska et al., 2002), possibly because of the presence of different morphotypes. *P. lanceolata* is a good candidate for a biomonitoring tool, as it is widespread, tolerant to increased levels of Pb (Wu and Antonovics, 1976), Sb (Baroni et al., 2000) and PAHs (Bakker et al., 1999). At the same time the plant can be vegetatively propagated (Wu and Antonovics, 1975) facilitating the avoidance of genetic variability in response to toxic substances. It is also particularly suitable for use in growth chambers and greenhouses, where it easily forms mycorrhizal associations (Walker and Vestberg, 1994). Research using *P. lanceolata* allowed the study of the toxicity of various industrial wastes (Orlowska et al., 2005). The arbuscular mycorrhizal richness, estimated in roots stained for fungal viability, seems to be equally sensitive to spore germination assays (Weissenhorn and Leyval, 1996). Additionally, several morphological changes of the mycelium in response to toxic metals could be tracked.

The use of ectomycorrhizal fungi in estimating substratum toxicity seems to be less useful. The plants inoculated with mycorrhizal fungi grew too slowly to be practicable; *in vitro* cultures of these fungi are not necessarily relevant to the field situation. Another type of monitoring of industrial wastes involves the analysis of the levels of Cd, Hg, Tl and Pb in the sporophores of edible fungal species. Different studies have shown a correlation between metal contents of fruitbodies and the distance from the source of pollution such as smelters and roadsides (Rühling et al., 1984; Rühling and Söderström, 1990). In the vicinity of Polish zinc wastes such studies are especially important, as people living in the area collect and consume fruitbodies of *Suillus luteus*. They are aware of the nature of these grounds, yet they cannot deny themselves the tasteful mushrooms. The analysis of heavy metal levels in *S. luteus* fruitbodies revealed exceptionally high concentrations of Cd and Pb. Cd is found in amounts often exceeding 20 mg/kg dry weight, corresponding to about 3 mg/kg fresh fruitbody mass. The consumption of mushrooms with the concentration of Cd exceeding 0.1 mg/kg of fresh mass constitutes a serious health hazard. In the case of fungi from the Chrzanow waste, this concentration is 30-fold exceeded. The World Health Organisation (WHO) indicates the admissible weekly Cd dose in food of 50 μg per kg body weight. Therefore, for a man weighing 80 kg this dose would be 0.6 mg per week, corresponding to 200 g of fresh mushrooms. Cd is relatively well accumulated in tissues, especially in liver, kidneys and bones. Its toxic effect consists of impairing liver function, hypertension, tumors and deformations of the skeleton due to changes in Ca metabolism. Very high levels of Cd (sometimes even higher than the above mentioned) were found in fruitbodies of many fungal species collected in the forests neighbouring the Zn-wastes. This results not only from the close vicinity of the wastes, but also from the geological structure (limestone rich in metal ores) and soil properties (low pH causing high availability of heavy metals for plants and fungi). Also the presence of Pb in fungal fruitbodies is dangerous. *S luteus* contains over 210 mg Pb per kg dry material, and *Chroogomphus rutilus* was demonstrated to contain as much as 610 mg, which corresponds to 28 and 81 mg Pb per kg fresh mass of these fungi, respectively. The threshold above which mushroom consumption is considered dangerous to human health was set to 0.5 mg Pb per kg fresh mass. Therefore it is enough to eat 20 g of *S. luteus* or 6 g of *C. rutilus* to reach this limit. Mushrooms from neighbouring forests contained significantly less Pb. The toxicity of Pb impairs the functioning of liver, kidneys, brain and bone marrow. In some localities also the level of Tl in fruitbodies may represent a potential health risk.

5. CONCLUSIONS

The exploitation of zinc ores near Chrzanow seems to be ended within a few years. After a long period of mining activities, the wastes will be left in a form of hills in the landscape. The adoption of the area, however, will not be easy. As long as the industry is operating, an almost sufficient amount of water is being pumped into the sedimentation tanks, the amount that is equal to rain water in this area, and this allows for the development of vegetation. The problem will start after this will be finished. Covering the waste with a layer of soil is too expensive to be feasible and various scenarios are proposed, such as covering the waste with material from other industrial deposits that again could result in creation of new risks. Whatever will be the case, the low-cost and effective phytoremediation of the area using mycorrhizal fungi that play the key role, is necessary. Other mycorhizosphere organisms that the fungi interact with should be also included. Concerning plants, it seems that the most promising candidates should belong to grass species, as they are fast growing and their root system is particularly effective in stabilizing the soil, together with carefully chosen mycorrhizal fungi. As it was proven that indigenous species are best adapted to the given site conditions, it seems that the best approach would involve isolating and culturing these strains. Optimizing practical application of phytoremediation may result not only in stabilization of the ground but can also create an interesting area that support the survival of rare and interesting plants, although this kind of depositions should be always carefully monitored and should never be considered as safe and stable.

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BIODEGRADATION OF PETROLEUM HYDROCARBONS BY KERATINOLYTIC FUNGI

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Abstract: The chapter reviews available data on the ability of keratinolytic fungi to remove hydrocarbons from different media and on the ecology of these fungi in oil-contaminated environments. In pure culture, these fungi were able to remove hexane, toluene, hexadecane, pristane and autoclaved crude oil from mineral media. The hydrocarbon removal process was much more effective when hair or peptone was added to the media. Thus, the process was dependent on fungal proteolytic or keratinolytic activity, specifically on the readily available protein content in the media. The ability for hydrocarbon removal was found to be species- and strain-specific. In pure culture, keratinolytic fungi removed polar products of petroleum biodegradation from the media. In a soil environment, the degradation process was slowed down due to the accumulation of these polar products.

Key words: keratinolytic fungi; oil hydrocarbon removal; survival; ecology; oil-contaminated environments

1. INTRODUCTION

Two major reasons to examine keratinolytic fungi in the environment can be named. First, the abundance of these microorganisms is observed in environments rich in keratinous remnants of human and animal origin and in other substrata needed for fungal growth, e.g., in soils of highly populated and animal-inhabited areas, sewage sludge and municipal waste (Garg et al., 1985; Deshmukh and Agrawal, 1998; Ulfig, 2000). Keratinolytic fungi play

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the main role in the decomposition of these substrata and can be used for biotechnological applications (Kushwaha, 1998; Onifade et al., 1998). Second, keratinolytic fungi display potential pathogenic properties to animals, including humans (Filipello-Marchisio, 2000). Therefore, studies of these fungi in the environment are of epidemiological importance. Keratinolytic fungi also occur in abundance in highly industrialized and polluted areas, in which organic and inorganic contaminants considerably affect microbial populations. Therefore, an essential problem is an evaluation of the effects of these contaminants, including oil hydrocarbons on fungal distribution in these areas. The role of keratinolytic fungi in biodegradation of these hydrocarbons is also to be explained.

Extensive studies have recently been carried out to explain the factors influencing keratinolytic fungi in the environment (Ali-Shtayeh and Jamous, 2000; Ulfig, 2000; Ulfig et al., 2003a). Special attention has been paid to those fungi inhabiting environments heavily contaminated with oil hydrocarbons. The present chapter reviews available data on the ability of keratinolytic fungi to remove the hydrocarbons from different media and on the ecology of these microorganisms in the above-mentioned environments.

2. KERATINOLYTIC FUNGI AS BIOINDICATORS OF TOXICITY AND BIOREMEDIATION PROGRESS

Ecotoxicity bioassays for bioremediation have comprised a great number of organisms (Salanitro et al., 1997), but the available literature indicates that microscopic fungi have not been used for toxicological studies during bioremediation. However, antifungal properties of oils, fatty acids, and some volatile compounds have been known for almost one hundred years (Garg et al., 1985). During biopile and column experiments within a bioremediation study at one of the Polish oil refineries, it was observed that keratinolytic fungi reacted to the changes in TPOC (Total Petroleum Organic Carbon that includes non-polar hydrocarbons and their polar derivatives) concentrations in leachates. This finding inspired us to examine the inhibitory effect of leachates on radial growth and dry weight (biomass) production of these fungi (Ulfig et al., 1998).

Based on the results obtained, the bioremediation process could be divided into three stages. The first stage included the first six months of the process and was characterized by a high inhibition of fungal growth. The second stage was three-month long and showed a considerable decrease of the inhibition. The last stage (after 9 months) was associated with low inhibition. The inhibition of fungal growth and dry weight production inhibition correlated with TPOC values in leachates (Figure 1). Polar compounds prevailed in these leachates. The dry weight production was more sensitive to the leachates than the radial growth of the fungi. It was evident that below certain TPOC levels (concentrations of polar compounds) the leachates stopped inhibiting the growth of fungi and started stimulating them to grow. Since the leachates were sterilized by autoclaving, the data did not include volatile hydrocarbons.

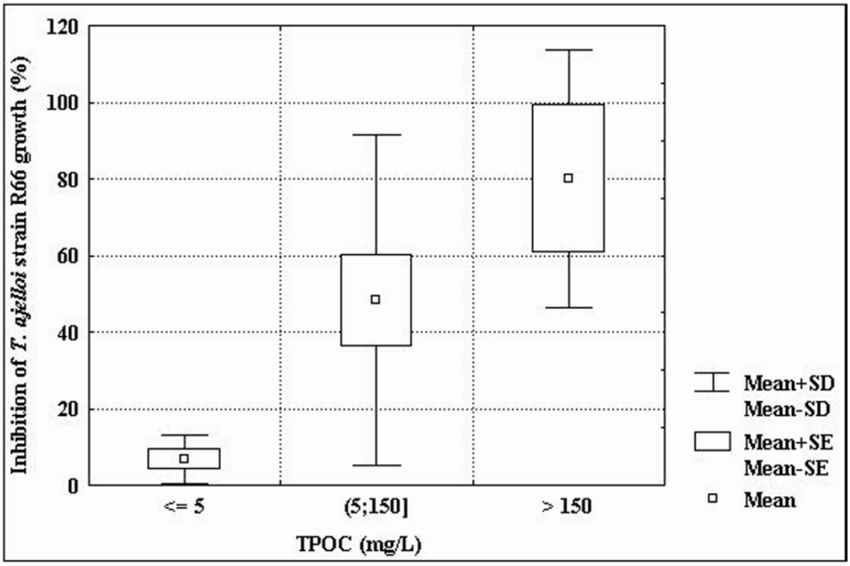

Figure 1. The relationship between the inhibition of the growth of the *Trichophyton ajelloi* strain R66 and TPOC concentration in leachate.

In another study (Ulfig et al., 2003b), keratinolytic fungi were found to occur frequently in a biopile with the geophilic dermatophyte, *Trichophyton ajelloi* (teleomorph *Arthroderma uncinatum*), as the predominating species. The fungal growth was dependent on the concentration of oil contaminants in soil. Negative correlations between fungal growth indices and TPH (Total Petroleum Hydrocarbons that includes non-polar compounds), TPOC and PAHs were found. No other data on the influence of PAHs on keratinolytic fungi were found in available literature. Overall, the keratinolytic fungi, especially *T. ajelloi*, were found to be a useful tool for a rough assessment of oil hydrocarbon contamination and associated bioremediation progress of soils heavily contaminated with hydrocarbons.

3. SURVIVAL AND VIABILITY OF KERATINOLYTIC FUNGI IN AUTOCLAVED CRUDE OIL

Geophilic dermatophytes possess the ability to survive for a long time in autoclaved crude oil (Ulfig et al., 2002). These fungi were also able to attack hair in contact with conidia suspensions in oil. The survival time and growth on hair varied between species and strains. Overall, *Trichophyton terrestre* displayed the longest survival time (>6 months) in oil and the best growth on hair, while *Microsporum* sp. only survived for ≤3 months in oil and had the worst growth on hair. In comparison with the dermatophyte growth on hair laid on soil, fungal growth on hair partly submerged in conidia suspension in oil was retarded for about one month. Subsequently, coating the hair with autoclaved crude oil limited the growth of *T. ajelloi*. Broadly, the geophilic dermatophytes isolated from a refinery site were found to be resistant to autoclaved crude oil (non-volatile hydrocarbons). The mechanism of this resistance is unknown.

4. THE INFLUENCE OF VOLATILE OIL HYDROCARBONS ON KERATINOLYTIC FUNGI

A study was performed to determine the effect of volatile oil hydrocarbons on keratinolytic fungi (Ulfig and Płaza, unpublished data). Petri dishes with a refinery soil covered by hair were placed in desiccators with open side tubes. On the bottom of one desiccator, dishes with crude oil were placed at the beginning of the experiment. In another desiccator, dishes with oil were replaced every week. A desiccator without oil served as control. The experiment lasted for two months. In the desiccator environment, BTEXs adsorbed on activated carbon were measured with a GC/MS method. In comparison to the control, the single oil application stimulated keratinolytic fungi to grow on hair. Subsequently, the repeated oil application considerably restricted the growth of the fungi, except for *T. ajelloi* strains (including the strain R66). These strains were found to be resistant to continuous high concentrations of volatile oil hydrocarbons. The mechanism of this resistance is unknown.

5. UPTAKE OF HEXANE AND TOLUENE BY KERATINOLYTIC FUNGI

Selected dermatophytes such as *Microsporum gypseum, M. canis, Trichophyton terrestre, T. ajelloi, T. mentagrophytes var. mentagrophytes* and *T. rubrum* and two other keratinolytic fungi, e.g. *Aphanoascus reticulisporus* and *Scopulariopsis brevicaulis* were surveyed for hexane and toluene uptake (Ulfig and Płaza, 2004). The strains examined were isolated from oil-contaminated sites and skin lesions. The closed serum bottle and headspace GC/MS methods were used. It was found that the average hexane uptake was comparable to the mean toluene uptake by the fungi examined. However, the mean dry weight production was much higher for hexane uptake. The highest hexane uptake was observed for *S. brevicaulis*, followed by *T. ajelloi, M. canis, T. mentagrophytes* and other species. Subsequently, the highest toluene uptake was observed for *T. terrestre*, followed by *S. brevicaulis, T. mentagrophytes, T. ajelloi* and other species. The lowest hydrocarbon uptakes were found in *A. reticulisporus*. The *M. canis* hexane uptake was much higher than its toluene uptake.

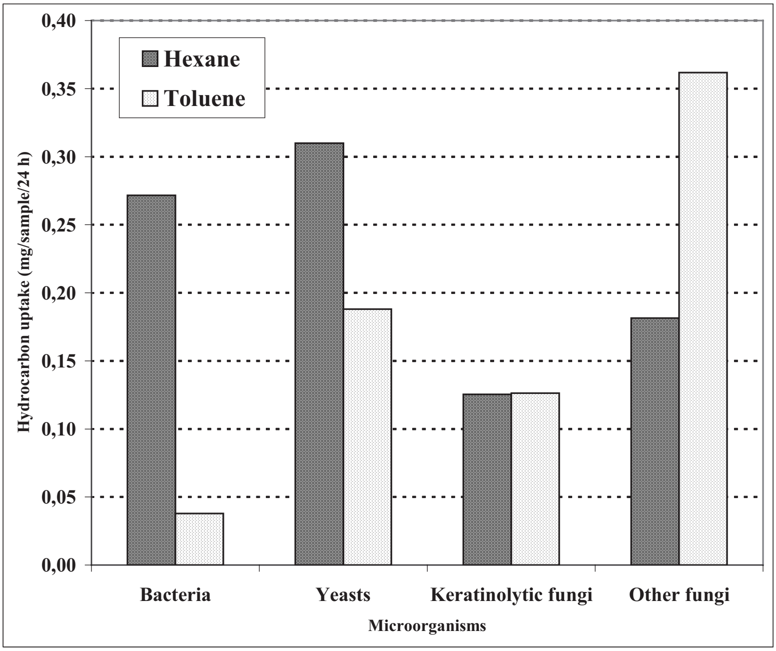

Figure 2. Hexane and toluene uptakes by bacteria, yeasts, keratinolytic fungi and other fungi.

Generally, the geophilic dermatophytes showed higher hydrocarbon uptakes than zoophilic and anthropophilic species did. The results were compared with the data obtained for other filamentous fungi, yeasts and bacteria isolated from oil-contaminated sites. Keratinolytic fungi had the lowest hexane uptake but the uptake of toluene by these fungi was higher than that of bacterial strains (Figure 2). Keratinolytic fungi produced much higher dry weight than the other microorganisms did.

6. REMOVAL OF OIL HYDROCARBONS BY *TRICHOPHYTON AJELLOI* STRAIN R66 DURING HAIR BIODEGRADATION

In a preliminary study (Ulfig et al., 2000), the efficiency of oil hydrocarbon removal from a mineral medium by the *T. ajelloi* strain R66 was determined.

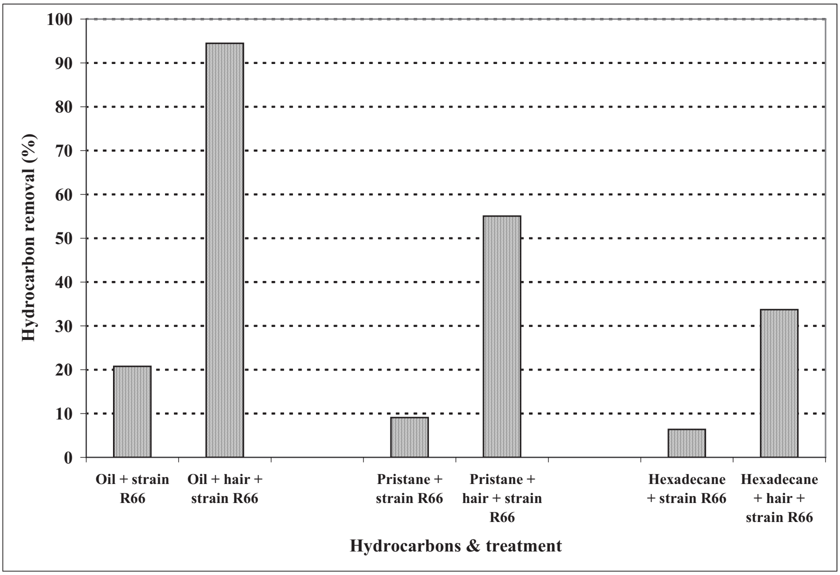

Figure 3. Hydrocarbon removal by the *Trichophyton ajelloi* strain R66 from the mineral medium supplemented or non-supplemented with hair.

The considerable amount of losses of pristane and hexadecane from autoclaved crude oil, were observed during the biodegradation of hair by this strain. Without an addition of hair to the medium, the strain did not decrease, or decreased to a small degree, the amount of these hydrocarbons.

The highest hydrocarbon losses were determined for crude oil, followed by the losses for pristane and hexadecane (Figure 3). The study confirmed the processes of proteolysis, sulfitolysis, and alkalization of the mineral medium during hair biodegradation. The presence of hydrocarbons made the process of hair biodegradation by the strain R66 more effective.

7. REMOVAL OF OIL HYDROCARBONS BY KERATINOLYTIC FUNGI DURING PROTEIN BIODEGRADATION

7.1 Pure Culture Experiments

The removal of autoclaved crude oil from mineral medium containing NH_4NO_3 supplemented or non-supplemented with hair by keratinolytic fungi strains was examined (Miksch et al., 2002). The strains were isolated from bottom sediments, sewage sludge and oil-contaminated soil and belonged to the species as follows: *A. reticulisporus*, *Chrysosporium keratinopilum*, *Chrysosporium* sp., *M. gypseum*, *T. ajelloi* and *T. terrestre*. The mean TPH and TPOC removals from the medium without addition of hair were 18 and 14.5%, respectively. The addition of hair to the medium did not significantly change the mean hydrocarbon removals, which were 18.6 and 17.1% for TPH and TPOC, respectively. Among the strains examined, the *T. terrestre* strain PS22 was exceptional. In this case, the addition of hair to the medium increased TPH and TPOC removals from 13.1 and 10.8% to 45.4 and 45.6%, respectively. A relatively high increase of TPH/TPOC removals after addition of hair to the medium was also obtained for some *M. gypseum* strains.

In a subsequent experiment, the removal of autoclaved crude oil from mineral medium containing peptone (4 g/L) supplemented or non-supplemented with hair (100 mg/sample) by keratinolytic fungi strains was determined. The mean TPH and TPOC removals from the medium without addition of hair were 53.4 and 54%, respectively. The addition of hair to the medium considerably increased the mean hydrocarbon removals, which were 71.8 and 71.7% for TPH and TPOC, respectively. The highest hydrocarbon removal from the medium without addition of hair was observed for *T. ajelloi* (67.2%) and from the medium with hair addition for *T. terrestre* (88.9%).

Two strains, i.e. *C. keratinopilum* PS14 and *T. ajelloi* PS16, were used for determination of the influence of increasing peptone concentrations (0, 2, 4 and 8 g/L) and hair addition (100 mg/sample) to the medium on fungal hydrocarbon removals. The results are illustrated in Figure 4. The strain PS14 increased TPH removal with increasing peptone concentrations. The addition of hair caused further THP removal increase up to the peptone concentration of 4 g/L. However, the statistically significant TPH removal increase caused by addition of hair was observed for the medium without peptone and at a peptone concentration of 2 g/L. The strain PS16 increased TPH removal up to the peptone concentration of 4 g/L. The addition of hair caused TPH increase at the peptone concentrations of 4 and 8 g/L. However, the differences observed were not statistically significant. The TPOC removals were found to be similar to TPH removals. The TPH and TPOC removals were positively correlated with the increase of NH_4-N (r = 0.83 and 0.86), dry weight (0.71 and 0.73) and pH (0.57 and 0.58).

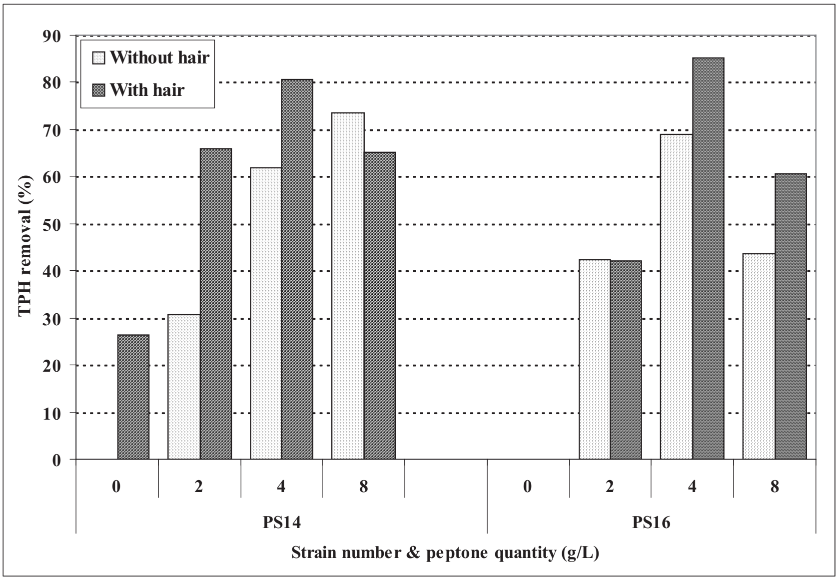

Figure 4. Oil hydrocarbon removal by the *Chrysosporium keratinophilum* strain PS14 and *Trichophyton ajelloi* strain PS16 from the media containing increasing peptone concentration supplemented or non-supplemented with hair.

The results confirmed that the oil hydrocarbon removal process was associated with fungal keratinolytic activity. Proteins (hair or peptone) were the main source of nitrogen, carbon and sulfur for the growth of these fungi, whereas oil hydrocarbons were the additional but essential source of carbon for some strains examined. However, the biochemical mechanism of this phenomenon requires explanation in further studies.

7.2 Soil Experiments

In a soil experiment, the influence of increasing autoclaved crude oil amounts (0, 0.5, 1, 2 and 5 mL per Petri dish) on the growth of keratinolytic fungi on hair laid on clayey, organic and sandy soils was examined (Miksch et al., 2002; Przystaś et al., 2002). TPH and TPOC removals from samples covered and uncovered by hair being decomposed by the fungi were also determined. *T. ajelloi* was found to be the only keratinolytic fungus in clayey and sandy soils and predominated in organic soil. The addition of 0.5- and 1-mL oil portions to the clayey soil stimulated *T. ajelloi* to grow on hair. Higher oil amounts restricted the growth of this fungus. The addition of oil (even in the smallest portion of 0.5-mL) to the organic soil restricted the growth of keratinolytic fungi. The addition of 5-mL oil portions to this soil eliminated the fungi. *T. ajelloi* grew in all Petri dishes with sandy soil. However, the amount of the observed mycelium decreased with increasing oil amounts added to this soil.

The TPH removal from the clayey soil covered by hair being decomposed by fungi decreased with increasing oil supplementation. In the organic soil, the highest TPH removal (89.7%) was observed in samples supplemented with 1-mL oil portions. This was also the highest TPH removal in the whole experiment. The TPH removals considerably decreased with increasing oil amounts added to the organic soil. In the sandy soil, the highest TPH removal was observed in samples supplemented with 1-mL oil portions. The TPH removals from the sandy soil decreased with increasing oil supplementation. Broadly, the TPOC removals were found to be lower than the TPH removals. This was well observed in clayey and organic soils. Except for the organic soil, the addition of hair did not significantly change TPH and TPOC removals from soils. The hair on the organic soil considerably decreased the TPOC removal. This indicated that polar products of the oil biodegradation process were accumulated in the samples. The acidification of the soils examined was also observed during a 5-month incubation.

In another soil experiment (Miksch et al., 2002; Przystaś et al., 2002), the hair cover on cattle farm soil generally decreased TPH and TPOC removals. The accumulation of polar compounds (TPOC) and acidification of this soil were again evident. It can be stated that both the factors slowed down the oil hydrocarbon biodegradation process in the soils examined.

8. CONCLUSIONS

The inhibitory effect of polar compounds (products of the oil hydrocarbon biodegradation process) in leachates on the radial growth and dry weight (biomass) production by *T. ajelloi* and the qualitative and quantitative composition of keratinolytic fungi can be used as indicators of bioremediation progress and associated contamination of a biopile soil with oil hydrocarbons. The geophilic dermatophytes possess the ability to survive for a long time (over six months) in autoclaved crude oil. These fungi are also able to attack hair in contact with conidia suspension in oil. The survival time and growth on hair varies between species and strain. Geophilic dermatophytes isolated from an oil-polluted site are resistant to non-volatile oil hydrocarbons. When applied once, volatile oil hydrocarbons (measured as BTEXs) enrich the composition of keratinolytic fungi in soil. However, continuous high volatile oil hydrocarbon concentrations inhibit fungal growth. Only some strains of *T. ajelloi* were able to survive long exposition to these compounds. No data on the impact of PAHs on keratinolytic fungi are available. The resistance of fungal strains to high oil hydrocarbon concentrations requires explanation in further studies.

In pure culture, oil hydrocarbon removal depends on fungal proteolytic or keratinolytic activity and is associated with high dry weight (biomass) production. The fungal ability for hydrocarbon removal from the medium during protein biodegradation is species- and strain-specific. The hydrocarbon addition to the medium stimulates, to a certain degree, fungal keratinolytic activity. These abilities can be used in biotechnologies, in which protein addition accelerates hydrocarbon biodegradation or *vice versa*. Proteins, including keratin are the main source of nitrogen, carbon and sulfur for the growth of these fungi, whereas oil hydrocarbons are the additional but essential source of carbon for some strains examined. The biochemical mechanism of this phenomenon requires explanation in further studies.

In pure culture, keratinolytic fungi are able to remove polar compounds from the medium. In soil, however, these compounds are accumulated, which may intoxicate the soil environment and slow down the hydrocarbon biodegradation process. The TPH removal decrease caused by addition of hair to the soil, accumulation of polar compounds, soil acidification and weak abilities of keratinolytic fungi to compete with other soil hydrocarbon degraders question a wide use of keratinous waste or biosolids containing high amounts of keratinous substrata, e.g. sewage sludge, for bioremediation purposes. Oil and keratinous substrata also support the growth of some strains of pathogenic *M. gypseum* in the environment.

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Management strategies for large-area contaminated sites

7.1. Integrated management strategy for complex groundwater contamination at a megasite scale
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INTEGRATED MANAGEMENT STRATEGY FOR COMPLEX GROUNDWATER CONTAMINATION AT A MEGASITE SCALE

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Abstract: To manage complex groundwater problems at the so-called megasites, a risk-based Integrated Management Strategy (IMS) of the European WELCOME project is currently applied to three megasites in Tarnowskie Góry (PL), Bitterfeld (D) and Port of Rotterdam (NL).

Key words: megasite, Integrated Management Strategy; Conceptual Model; Risk Management Zone; Risk Management Scenario

1. INTRODUCTION

Complex environmental problems exist at the so-called megasites, i.e. areas with multiple contaminant sources related to (former) industrial activities, with a considerable impact on the environment, through groundwater, surface water and/or air migration. A risk-based approach can be developed by using **Integrated Management Strategy (IMS)** of the EU WELCOME project that includes stakeholders' participation, compliance with the EU legislation – the Water Framework Directive (WFD) and GroundWater Directive (GWD), a megasite Conceptual Model (CM), delineation of a Risk Management Zone (RMZ) using planes of compliance and, finally developing a Risk Management Scenario (RMS). The IMS is currently applied to three EU megasites contaminated with heavy metals and

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persistent organic pollutants: **Tarnowskie Gory (PL), Bitterfeld (D)** and **Port of Rotterdam (NL).**

2. IMS DESCRIPTION

The IMS is a stepwise approach to establish integrated risk-based management plans for contaminated megasites (www.euwelcome.nl). It is aimed to guide the user through the whole megasite management process (Figure 1): from the initial screening to the final definition of the remediation scenarios and the long-term site management plan. It assists to distinguish the areas of the megasite with the highest risk, set up priorities for risk reduction, decontamination, and consequently related investments based on risk assessment. This often allows for minimizing the costs and maximizing effectiveness of the project by relocating investments into the most endangered areas of the site.

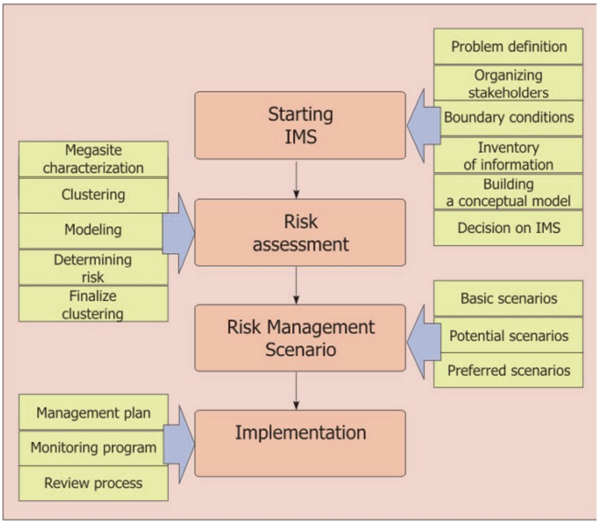

Figure 1. Structure of the Integrated Management Strategy (IMS).

To apply the risk-based management at the specific megasite, the user is advised to follow the stepwise sequence given by the system (Malina, 2004a). The system provides information and guidance but does not interact with the user. This implies that the user is producing the output himself, and evaluates it based on the guidance provided. The IMS is structured as a multi-level system. It provides sufficient information to the technical megasite managers/site owners/stakeholders to judge upon applicability of the system at the particular megasite. It gives detailed information for the technical experts/consultants, as well; to carry out specific tasks needed to establish an integrated risk-based management plan.

3. IMS CASE STUDIES

3.1 Integrated Strategy at the Tarnowskie Góry Megasite

At Tarnowskie Góry (TG) in Poland, the soils and the Quaternary (Q) groundwater are contaminated with heavy metals and boron, originating from uncontrolled waste disposal in the vicinity of the chemical plant closed down in 1995 (Malina, 2004b). This contamination is hazardous to the Triassic (Tr) reservoir, the main source of potable water, and it may potentially affect the population of more than 600,000. The general management goal is to protect the drinking-water supply function of the Tr reservoir. To-date site remediation is based on construction of a controlled landfill (an area of 16 hectares), where waste, debris and contaminated soils are dumped. Due to complexity of the site conditions, contaminant characteristics, organizational and regulatory aspects, and/or considerable costs, an integrated risk-based approach is recommended to manage the risks for the defined receptors.

The CM includes the primary (hazardous waste deposits) and secondary (contaminated soils and Q sediments) sources of contamination. Contaminants are leached out from the waste heaps and infiltrate through the unsaturated zone to Q aquifers, accumulate there and/or migrate further. Migration to the Tr formations may occur through the so-called hydrogeological windows (i.e. discontinuities of overlying not-permeable clay layers). Moreover, a direct contact between waste materials and the Tr formations observed in some areas may result in direct groundwater contamination. Based on: (i) frequency of occurrence, (ii) fate, mobility, persistence and abiotic natural attenuation (NA), (iii) toxicity and impacts to the receptors, and (iv) availability of data, the priority contaminants (As, Ba, B, Cd, Sr and Zn) were selected. Three major risk clusters, i.e. zones, where the risks can be managed in a defined way, were defined that are oriented on receptors, pathways, boundary conditions and stakeholders' interests. Within each cluster, the primary and secondary contamination sources were recognized. Based on the existing and/or predicted groundwater contamination, the RMZ was delineated, i.e. an area, where the requirements of the EU WFD (2000) may not be fulfilled for a defined period of time. The RMZ provides space and time to manage risks for receptors in a cost-effective way. Planes of compliance were also derived (Figure 2), i.e. the horizontal or vertical borders, at which a certain concentration or a mass flux have to be achieved, to comply with the site-specific threshold values.

The basic RMS was developed according to the possible measures: (i) source-oriented, i.e. capping or removal of heaps (primary sources), contaminants immobilization in soils and Q deposits (secondary sources), (ii) pathway-oriented, i.e. internal/external hydraulic barriers, and (iii) receptor-oriented, i.e. monitoring and development of a specific water extraction regime at the receptors (wells).

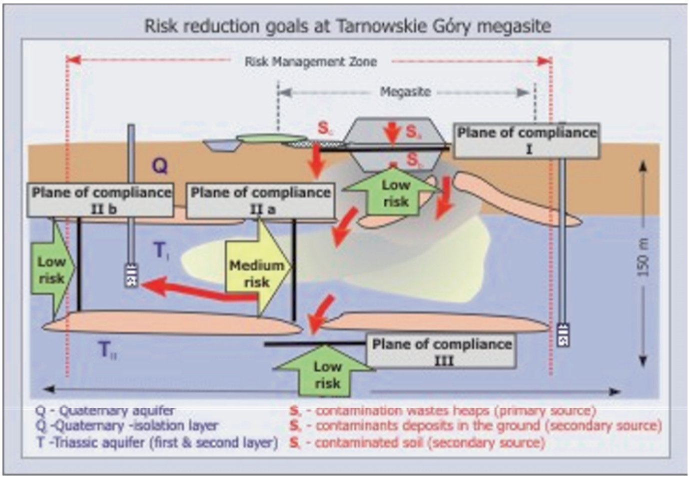

Figure 2. The risk reduction goals for the TG megasite.

The effectiveness of the basic RMS in fulfilling the management goals was analyzed based on: (i) containment of the boron plume within the RMZ (defined by the 1 mg B/dm^3 concentration contours in groundwater), (ii) stabilization of the plume within the current boundaries, and (iii) maximum possible reduction of the plume within a moderate time frame.

The boundary conditions were revised, and the management plan accepted by stakeholders includes: (i) construction of a controlled landfill (area: 16 ha, capacity: 1.6 million m^3) for hazardous waste deposition, in a way that complies with the legal Polish and EU requirements, (ii) revitalization of the Stoła River valley, (iii) reclamation of previous dumps (ca. 50 ha) with allocation of ca. 10 ha for the further use, and (iv) continuous long-term monitoring of soil, water and air. The results of simulation studies show that waste removal and landfilling should have a moderate effect on improving groundwater quality in a short (30 years) and medium (60-80 years) time-frames. It guarantees that some of the risks at the surface are moderated or eliminated, whereas the risk from the secondary sources of contamination is still present.

Three potential RMS were built up: S1 – controlled natural attenuation NA (source removal + monitoring/control of the hydrological regime), S2 – ctive groundwater remediation (source removal + groundwater clean-up + monitoring), and S3 – engineered natural attenuation NA (source removal + increased groundwater extraction within the RMZ + monitoring).

The final RMS was not selected by the group of stakeholders (GOS) due to uncertainties in boundary conditions. Currently, scenario S1 is implemented; however, two other scenarios are not excluded. The established RMZ is formally included in the watershed management plan realized jointly by the local and regional administration, and the chemical plant liquidator. Monitoring focuses on the Tr aquifers to trace migration of contaminants (pathways) from the dumping sites (primary sources) and Q sediments (secondary sources) to the water wells (receptors).

3.2 Integrated Management at the Bitterfeld Megasite

The Bitterfeld (BTF) megasite is located in the State Saxony-Anhalt in Germany, and characterized by over 100 years of open cast lignite mining activities and chlorine chemical industry. Due to the chemical production and improper dumping of industrial wastes, a large-scale groundwater contamination occurred, in combination with a rising groundwater table after closing down of the mining activities. More than 100 million m^3 of groundwater have been polluted by numerous contaminants and, therefore constitute a source themselves (Grossmann et al., 2003).

As complete clean-up of the megasite is neither technically nor economically feasible, according to the legal requirements given by the WFD, thus an integrated risk-based approach was applied. This included the delineation of a RMZ, comprising the industrial area and the surrounding impacted groundwater body, followed by determination of contamination spreading from the source area to the nearby main receiving stream Mulde, including already affected receptors, as e.g. the town of Bitterfeld. The RMZ comprises an area of approximately 48 km^2 leaving time and space for an effective and cost-efficient management of the contaminated groundwater.

Initial boundary conditions included: legal requirements, financial options and the future use of the site. The participation of numerous stakeholders from three main domains: mine site re-habilitation, site remediation and re-development of industrial activities, involved in the megasite management, was successfully organized and managed by regular meetings.

To support the development of the area, which suffered from the economical breakdown as result of the reunification, the entire megasite was generally exempted from remediation costs by means of a special law. Finances provided by the federal government to restore the megasite for a further industrial use, are administered by the specially founded State Authority for Exemption from Residual Pollution Responsibility (LAF).

To facilitate the technical management, the megasite was structured into five manageable units, the so-called risk clusters. They were delineated comprising areas of similar features with respect to contaminants (sources), hydrogeological conditions (pathways) and affected receptors (Figure 3).

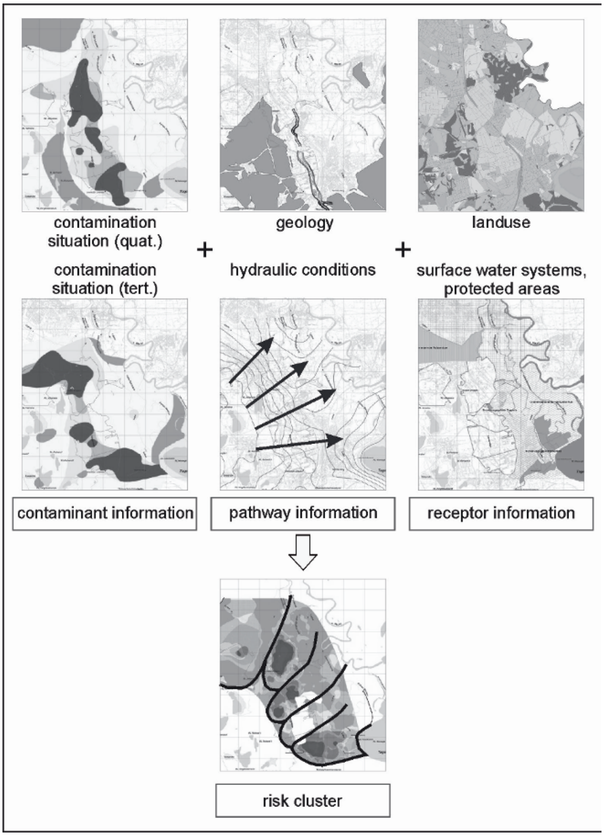

Figure 3. Formation of risk clusters at the BTF megasite.

As a result of risk assessment based on clusters and fate and transport (F&T) modeling, requirements for safety and risk reduction measures were derived, and risk reduction objectives defined as follows: (i) prevention of a further expansion of the contaminated groundwater beneath the industrial area, (ii) protection of the unpolluted groundwater outside the RMZ, (iii) protection of the receptors inside the RMZ, such as humans (residential, industrial, protected areas), water-dependent ecological systems, and (iv) long-term reversal of the contamination.

The areas affected by acute risks were subjected to emergency measures, particularly 'pump and treat', to maintain the depth to groundwater table that, as a consequence of the closure of mining activities, rose significantly.

RMS consists of technically feasible and cost-efficient measures to achieve the risk reduction objectives. Additional factors, such as the efficiency of measures, as well as infrastructural and political aspects are taken into account when selecting the preferred RMS for the clusters. The first selection of RMS, currently carried out for each cluster, was determined in the form of the so-called remediation framework concept (RFC) that will be further elaborated, leading to the optimized concept for the entire megasite.

The decision-making process is driven by the hydraulic conditions that are to be managed continuously, particularly since the flooding in 2002. Therefore, the RFC for the megasite focuses on hydraulic measures in combination with the enhanced NA ('hydraulically controlled bioreactor') to protect the groundwater (Kaschl et al., 2002, Wycisk et al., 2003, Heidrich et al.*,* 2004a,b). Additional measures are implemented for protection of receptors according to determined risks. The RFC for groundwater protection comprises four phases: (i) hydraulic outflow measures, (ii) hydraulic outflow and inflow measures, (iii) source remediation, and (iv) hydraulically controlled bioreactor. Neither all phases are identical in all clusters, nor are they implemented in each cluster. They depend on the degree of contamination, the existence of affected receptors and, hence on the priority of risks within the respective clusters. They constitute the framework, which is subjected to continuous optimization based on the increasing knowledge about the megasite, and particularly the results of the more detailed models. In combination with the research activities, the RFC will then be specified, including the implementation of complimentary measures (e.g. innovative technologies) at specific areas.

The development of the RFC, as well as the implementation plan, are discussed and confirmed by all stakeholders involved. As a result of the risk-based approach, the remediation concept was developed for soil and groundwater separately. The development of the RFC for the groundwater is an ongoing process since long-term measures are elaborated in parallel to the implementation and optimization of emergency measures.

The megasite monitoring is carried out twice a year with regard to groundwater quality and quantity, including the collection of samples from approximately 400 monitoring wells. Additionally, the surface water monitoring is implemented twice a year (regarding water quality) and monthly (concerning water quantity).

The entire megasite management will be implemented and organized based on a management plan. The management process will be continuously reviewed, and the results of the implementation of the management plan will be continuously verified and adjusted.

3.3 Application of IMS at the Rotterdam Megasite

The Port of Rotterdam (PoR), with its petrochemical contamination, is an example of point-source contamination at a megasite scale. The harbor itself, and industrial complexes, are situated at the delta of the rivers Rhine and Meuse, and covers an area of 10,500 acres. It is one of the world's largest harbors, with diverse activities including the transshipment and processing of bulk goods (oil, chemicals, coals and ores). These activities resulted in soil and groundwater contamination to such an extent that a conventional site-specific approach for clean-up is not feasible (Malina et al., 2004). It is disproportionately expensive (>35 M€/year), and environmental objectives will not be achieved. Instead, it is expected that the IMS offers a better solution for the contamination problems.

Application of the IMS requires the involvement of stakeholders (from the public and private parties): PoR, Environmental Agency for Rotterdam Region (DCMR), Ministry of Public Health, Spatial Planning and Environment (VROM), and industry (united in the interest group Deltalinqs). The steering group is controlling the progress of the project and makes strategic decisions based on balancing economical vs. environmental interests, and (future) region development**.**

The entire PoR is considered as one area that combines all contaminated sites, provides time and space for NA processes and prevent that contaminants reach the borders of the harbor area. Based on the CM, the RMZ was delineated by means of planes of compliance, i.e. boundaries between sources and receptors to be protected. The 1st plane of compliance marks the direct influence of shallow groundwater contamination to the surface waters (harbor). Two groundwater receptors were identified: the aquifers directly below (2nd plane of compliance) and outside (3rd plane of compliance) the harbor area (Figure 4).

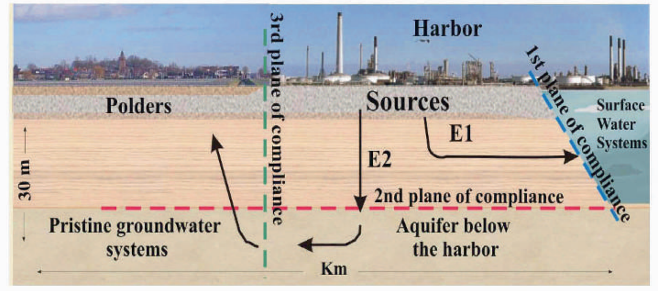

Figure 4. Plane of compliance at the PoR megasite.

The megasite characterization provides information to assess the contaminant distribution, hydrogeology and NA potentials, required to predict the impact of contaminants to the receptors, and to determine the effectiveness of the RMS. F&T modeling was used to predict the impact of contaminants to the surface waters and deeper groundwater systems. By comparing the impacts to the risk-based standards and stakeholders' objectives, the risk could be assessed. It is directed to an autonomous situation without risk reduction measures, considering the impact to: (i) surface water (1st plane of compliance) - the contaminant flux from the groundwater is relatively limited compared to other emission sources, and the total concentration within the surface water remains well below (negligible risk) standards, (ii) aquifers (2nd plane of compliance) - currently considerable (8% of the aquifers impacted), and predicted to increase gradually (up to 12% in 2040), and (iii) adjacent groundwater systems (3rd plane of compliance) - currently limited (3% of the border impacted) but a strong increase is forecasted (up to 8% in 2030 and 10% in 2050).

To assess the feasibility of RMS, several source-, pathway- and receptor-oriented management options were defined, and worked out separately, in terms of the risk reduction and costs for different ambition levels, which are related to the desired efforts. The higher is the ambition level, the higher risk reduction and corresponding costs that are required. The timeframe for risk reduction and reversal trends, as required by the GWD, are crucial for making decisions. Based on the: risk reduction degree, timeframe to achieve the risk reduction/reversal trends, and corresponding costs, the GOS decides upon effectiveness of individual and combined measures. This leads to the: (i) source-, pathway- and receptor-oriented measures (basic and combined scenarios), and (ii) assessment of costs effectiveness (risk reduction predicted with a model, costs, fulfillment of boundary conditions and criteria of stakeholders, compliance with the EU legislation.

None of the basic RMS alone allows for the achievement of environmental objectives as formulated by the GWD, as well as the fulfillment of obligations for individual sites. Therefore, a combination of basic RMS is proposed for the megasite that meets with the main objectives and will cost ca.15 to 20 M€ per year.

The risk reduction achieved by the combined measures at 2nd and 3rd planes of compliance is compared to the autonomous situation. Besides, the absolute risk reduction and the time-scale are relevant. Uncertainty ranges in the risk reduction are explicitly indicated at the 25th, 50th and 75th percentile. This puts the results in the right perspective for a sound interpretation and decision-making.

The total clean-up of the megasite in 25 years would cost 2-3 billion €, i.e. 100 M€ per year, when using conventional measures (‘dig-and-dump’ and/or ‘pump-and-treat’). This is neither technically nor economically possible due to heavy industrial facilities at operation at the surface, nor it is acceptable from the general sustainability principles viewpoint. 100 million € per year could be indicated as disproportional when comparing with the current costs of soil and groundwater management (ca. 10 M€ per year). Three combined scenarios were developed that are technically and economically applicable, i.e. a factor of five less costly than the total clean-up. A relatively high contribution of ‘pump and treat’ (3–6 M€ per year) could be partially replaced by more effective monitored NA.

To assess the cost-efficiency of the combined strategy, the costs versus the risk reduction/trend reversal achieved needs to be evaluated. In addition, other criteria (i.e. liability obligations, public perception) may play also an important role in the selection of the preferred RMS. As experienced at other megasites, a purely risk-based approach is insufficient for an effective management plan, whereas the IMS takes the other factors also into account.

4. CONCLUDING REMARKS

The IMS is generic (attaching to national and EU regulations) and flexible (allowing for tailor-made, region-specific and iteractive approaches). It includes stakeholders’ participation, and is based on natural science principles (establishing trends and appropriate measures using risk assessment procedures, F&T models, and taking into account NA and immobilization processes). It includes costs indications, based on tools and engineering know-how, and thus offers means to address the proportionality principle as mentioned in the EU and many national directives. The IMS and tested case studies may be a basis for further technical detailing of the GWD with regard to management contamination at a megasite scale.

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MANAGEMENT OPTIONS FOR REGIONALLY CONTAMINATED AQUIFERS: A CASE STUDY AT BITTERFELD, GERMANY

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Abstract: Large-scale contaminated megasites like Bitterfeld in eastern Germany are characterized by a regional contamination of soil, surface water and groundwater as a result of a long and varied history of chemical production. While the contaminants in soils and sediments mostly represent a localized problem, pollutants in groundwater may spread to uncontaminated areas and endanger receptors like surface water and drinking water wells according to the site-specific hydrologic regime. From the toxicological point of view, the contaminants at the Bitterfeld megasite represent a dangerous cocktail of various harmful substances coming from a multitude of sources. Appropriate remediation techniques must be able to remedy the specific problems arising from 'hot spot' areas within the megasite in addition to preventing a further extension of the contaminated zone towards uncontaminated compartments. Therefore, a combination of specifically designed remediation technologies based on the pump and treat principle with *in situ* technologies, such as reactive walls and monitored/enhanced natural attenuation, is necessary to efficiently address the miscellaneous challenges at this megasite. In this paper, the currently known contaminant distribution, the associated problems for human health and the environment and possible remediation strategies are presented for the Bitterfeld megasite.

Key words: contaminated megasite; "hot spot" areas; contaminant distribution; polluted aquifer; remediation strategies

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1. INTRODUCTION

As a legacy of intensive use for industrial production, mining, etc. as harbors or military areas in the past, we are faced today with the existence of many areas that have been contaminated on a regional scale (WELCOME-Project, 2001). These so-called megasites represent a complex problem for the respective site owners, local authorities and involved environmental consultancy companies. The development of appropriate remediation strategies is complicated by the existence of extensive residential areas close to the contaminated areas, whose occupants may be exposed to short-and long-term health risks. In addition to the direct risks involved for humans and the ecosystem stemming from the large amounts of highly contaminated soil and groundwater at the site, a widespread diffuse pollution may result from the transport of contaminated sediments in surface water or wind displacement of dust particles carrying contaminants. The effect the contamination will have on the surrounding non-contaminated aquifers, surface water and eventually marine ecosystems are, therefore, dependent on the position of the megasite in relation to the river system (spring, stream and delta). Due to the large amount of contaminants that have entered the soil and the groundwater at megasites, it must be assumed that these sites will emit pollutants for a long time to come, if preventive measures are not undertaken. On top of that, a usually untraceable site history of contaminant inputs in addition to a large variety of contaminants of different chemical properties complicate the assessment of potential effects and represent a challenging situation for the remediator. Basic research in the field of groundwater remediation has in the past been mainly restricted to single-source contamination represented by single contaminant plumes, while mixtures of pollutants, which are typical for the situation at megasites, and possible synergistic and antagonistic effects, have so far not been examined to the necessary detail.

Since a complete cleanup of a megasite including the removal of the contaminated source areas in the groundwater is neither technically nor economically feasible within an intermediate timeframe (30 years), an effective remediation strategy must focus on risk minimization. Simple relocation and use of restriction measures are in many cases not a desired option for the whole area of a megasite, due to its size and the often high land reuse value.

An example for such a megasite is Bitterfeld region in Germany. A variety of contaminants, some of which are extremely hazardous for human health, besides being highly mobile and persistent, are found in the groundwater today after a long history of chemical industry and lignite mining (Figure 1) (for details see e.g. Heidrich et al., 2004a). Halogenated hydrocarbons are the dominant substances of concern, as a result of main production sectors in Bitterfeld: chloro-organic chemistry and manufacturing of pesticides and dyes (Chemie AG Bitterfeld Wolfen, 1993; Walkow et al., 2000). Other compounds generally found in the groundwater include simple aromatics (benzene, toluene, ethylbenzene and xylene (BTEX)) hexachlorocyclohexanes (HCH), polychlorinated biphenyls (PCB), organic phosphates and sulfur compounds, while a variety of other substances are found only locally.

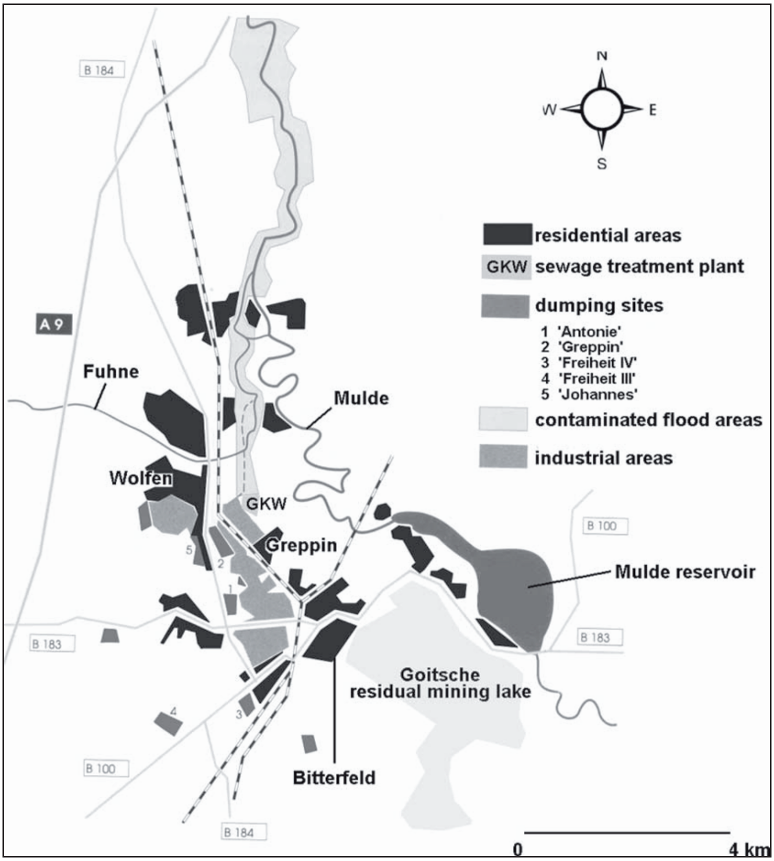

Figure 1. Schematic overview of the Bitterfeld megasite (modified after GICON, 2004).

Most of the raw materials, end- and side-products of the chemical industry in addition to their metabolites can be found in the groundwater today. In some cases, the breakdown products of degradation reactions in the groundwater are more toxic than the initial substances, such as is the case for vinylchloride, the product of anaerobic biodegradation of chlorinated ethenes, which is found in extremely high concentrations in the aquifer (Table 1).

Table 1. Concentration ranges of the most relevant contaminants in the Tertiary aquifer of the southeastern outflow in Bitterfeld between the industrial park and the Goitsche mining lake.

Substance	Concentration (µg/L)
Chlorinated aliphatics	
Trichloromethane (TCM)	680–77000
1,1,2,2-Tetrachloroethane (PCA)	110–30000
cis-Dichloroethene (cis-DCE)	220–23000
Trichloroethene (TCE)	350–21000
1,1,2-Trichloroethane	320–15000
Dichloromethane	930–14000
Tetrachloroethene	2500–12000
trans-Dichloroethene	410–5300
1,2-Dichloroethane	1000–4100
Vinylchloride (VC)	30–4100
1,1-Dichloroethene	600–990
Chlorinated aromatics	
Monochlorobenzene (MCB)	40–23000
BTEX	
Benzene	130–8200

The affected zone in the Bitterfeld groundwater may be described as a 'contaminated zone' resulting from an overlap of many different contaminant plumes coming from numerous contaminant inputs, which differ spatially and chronologically (Figure 2). These pollutants can be found dissolved in groundwater, but are also present in phases, which function as secondary contamination sources.

Investigations within the SAFIRA project (German acronym for: 'Sanierungsforschung in regional kontaminierten Aquiferen' [remediation research in regionally contaminated aquifers]) at the Bitterfeld site have shown large spatial differences in the quantitative and qualitative distribution of the organic contaminants in addition to variations over the aquifer depth (Weiss and Merkel, 2001). The contaminant profile demonstrates a strong vertical stratification and a locally heterogeneous distribution of contaminants in the groundwater, so that concentrations of contaminants may fluctuate widely from one monitoring well to another (Weiss et al., 2001; Merkel et al., 2001; Weiss et al., 2002).

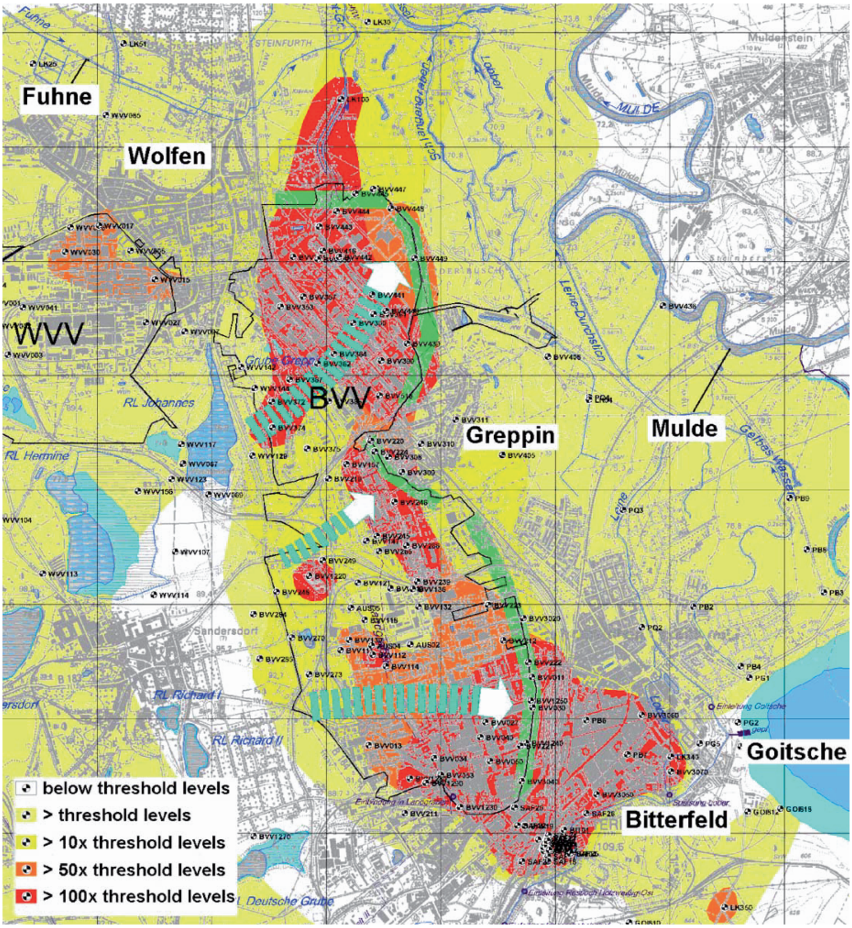

Figure 2. Contaminant levels in the Quaternary aquifer at the Bitterfeld megasite. Arrows indicate prevalent groundwater flow directions, while the green areas indicate the pumping barriers installed to contain the contamination. WVV: Wolfen industrial area, BVV: Bitterfeld industrial area.

2. REMEDIATION STRATEGIES FOR RISK MINIMIZATION

The regional extent of contamination of soils and groundwater at the Bitterfeld site prevents the adoption and implementation of a simple risk management strategy. A complete cleanup of the groundwater is neither economically feasible nor technically possible within a reasonable timeframe. Hence a remediation strategy is needed taking into account the direct risks for human health, the ecosystem and other so-called ‘protectable goods’ as well as the needs for industrial re-development of the region.

In order to provide cost-effective remediation schemes, efficient monitoring strategies need to be developed. These strategies include passive sampling methods such as time-integrating sampling devices (Grathwohl, 1999) for the assessment of contaminant fluxes over longer periods of time. In addition, depth-specific information on contaminant distributions is required to efficiently place active remediation measures or permeable reactive barriers (Schirmer et al., 1995; Nilsson et al., 1995).

To stop the uncontrolled spreading and extension of the contaminants in the groundwater towards uncontaminated areas and eventual receptors like the surface water, a common approach consists of pumping of the contaminated water and treating it (‘pump-and-treat’). The treatment steps consist mostly of stripping of volatile compound and subsequent adsorption on activated carbon; the treated water is then discharged into the recipient. If the quantity of water pumped is large enough, the groundwater flow regime may be altered and a further expansion of the contaminated zone avoided. However, this approach requires that relevant technologies exist for treating the particular contamination encountered at the site. A simple ‘pump-and-spill’, meaning a disposal of contaminated waters without adequate treatment, is certainly not acceptable from the quality of the surface waters viewpoint, and involves an additional potential risk of human exposure and an accumulation of pollutants in the environment. Currently, ‘pump-and-treat’ measures are being employed and their expansion considered at the Bitterfeld site within the framework of the Ecological Mega Project Bitterfeld–Wolfen (GICON, 2004). Barriers consisting of pumping wells are used to stop the groundwater flow in northern (N), northeastern (NE) and eastern (E) directions. The NE barrier has been in operation since 1994, using 20 pumping wells to extract a yearly amount of 1.3×10^6 m^3 contaminated water from the Quaternary aquifer (Lücke, 2002). This barrier prevents the spreading of contaminated plumes towards the flooding area of the Mulde river. A second barrier of pumping wells in the Greppin area is operating (Lücke, 2002), with a main aim of preventing the flooding of basements in residential areas and volatilization of contaminants from near-surface groundwater with inherent toxicological effects. The E barrier is intended to reduce the contaminant plumes moving towards and under the city of Bitterfeld in vertical and horizontal direction: 20–25 pumping wells with the capacity to extract 3.8×10^6 m^3 of water (Lücke, 2002). The water extracted in all pumping wells is divided into three categories, depending upon contamination: uncontaminated water is channeled directly into the

Mulde river, contaminated water with an halogenated organics (AOX) ≤3 mg/L is treated in the on-site wastewater treatment plant, while water with an AOX > 3 mg/L must be treated prior to discharge into the plant . Especially for the Greppin site, the high contamination and variety of contaminants (mostly chlorinated aliphatic compounds like PCA, TCE, VC in addition to MCB) requires the use of novel technologies. A promising approach, that has been successfully tested in a pilot plant on-site, makes use of sequential steps employing catalytic, reactive and sorptive mechanisms to decrease the AOX below 3 mg/L (Weiss et al., 2002). Such tailored technologies, which have to be adapted to the particular situation at a certain 'hot spot' of a megasite, will usually employ an efficient combination of several methods to reach the remediation goal.

A second strategy to contain the flux of contaminants in 'hot spot' areas of a megasite is the use of *in situ* technologies. These have the advantage of eliminating the problem of dealing with extracted contaminated groundwater on the surface. Specifically, for the southeast on the Bitterfeld site with high levels of MCB and other chlorinated compounds in the groundwater, appropriate technologies have been extensively investigated in the SAFIRA-project (Weiss et al., 2001). The most promising approaches involve the use of combined methods merging the adsorption capacity of activated carbon with the reducing potential of Fe^0 and oxidizing effects of oxygen-release compounds (ORCs), and the stimulation of the inherent biodegradation potential of the indigenous microbial population (Weiss et al., 1999). These technologies, which have so far been studied in pilot plant reactors fed directly by groundwater from the Quaternary aquifer, may be up-scaled and used in the so-called 'sorptive barriers' or '(im)/permeable reactive barriers' installed in the aquifer (a schematic illustration of the latter is given in Figure 1A). Reactive barriers are located perpendicular to the main direction of contaminant flow and decrease the contaminant loading by, e.g. adsorbing reactions (e.g. activated carbon) or through redox reactions (Fe^0, ORC) (e.g. Gillham and O'Hannesin, 1994; Teutsch et al., 1996; Benner et al., 1997).

A variation of reactive barriers is the funnel and gate-approach, where the contaminant plume is 'funneled' by impermeable funnel walls towards the reactive 'gate', the zone where the contaminants are broken down or adsorbed (Gillham and O'Hannesin, 1994).

For those areas of a megasite lying downstream of the main contaminant zone, monitored natural attenuation (MNA) may prove to be a valuable, additional remediation tool to be included in the overall strategy (Figure 3 B). MNA is based upon a variety of physical, chemical or biological processes that, under favorable conditions, act without human intervention to

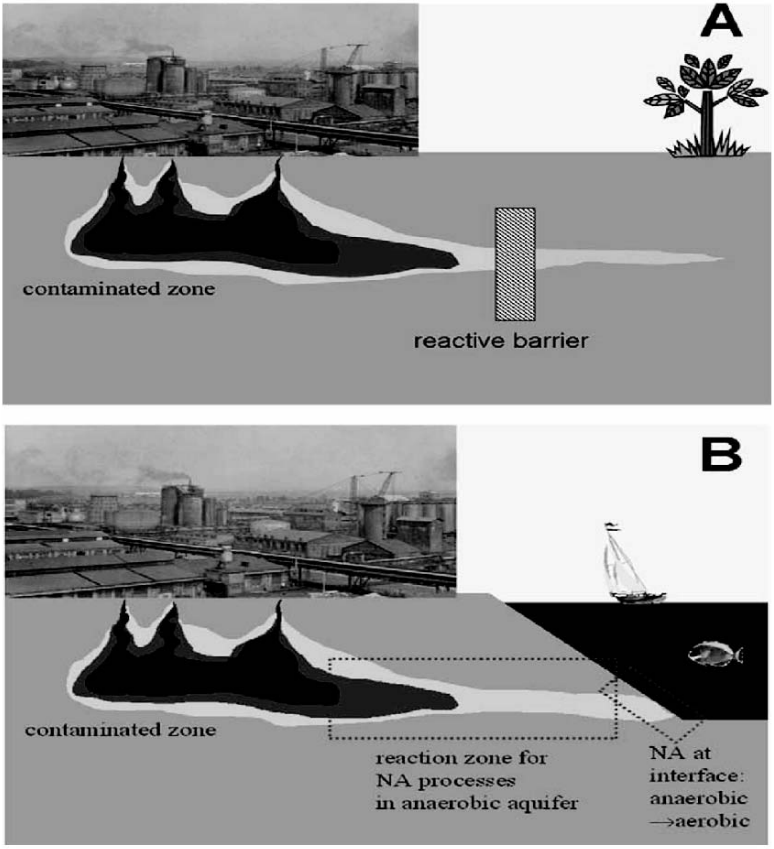

Figure 3. In Situ remediation technologies employed at certain areas of a megasite. A: reactive barrier, B: monitored natural attenuation.

reduce mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater (NRC, 2000). The establishment of the site-specific attenuation potential of such processes (‘NA potential’) is based upon the ‘three lines of evidence’ approach (US EPA, 1999). These lines of evidence include key footprints of natural attenuation (NA) processes from geochemical data, substantiating the reduction of contaminant concentrations and/or mass along the flow path as well as microbiological data giving evidence of biodegradation processes (US EPA, 1998; AFCEE, 1999; NRC, 2000). In addition, modeling becomes increasingly important for an approximation of the relevance of NA processes, since carrying out an extensive, detailed investigation and characterization of a megasite like Bitterfeld is often difficult.

After sufficient NA rates have been confirmed on site, long-term monitoring must ensure the persistence and relevance of these rates to minimize all relevant risks in order to guarantee a permanent protection of man and the environment. For instance, a growing contaminated zone threatening to spread to uncontaminated compartments and/or to be released to air and surface water, would certainly not constitute an appropriate place for MNA as an unassisted remediation measure. MNA must be able to contain all contaminants including persistent and mobile contaminants present in that area of the megasite, where it is employed as the method of choice, and no increased exposure to humans is permissible. Hence, MNA will be suitable only at defined areas of a megasite and, thus will be employed as a complementary strategy to other active remediation measures such as pump-and-treat or *in situ* reactive walls. The latter would be used to stop the contaminant flux in 'hot spot' locations, while MNA would prevent further spreading of contaminants at the fringes of the contaminated zone (refer to Figure 3 B). Currently, two areas in Bitterfeld are investigated regarding their suitability for the application of MNA (Heidrich et al., 2004b). In the north, in the future wetland area between the Wolfen industrial area and the surface water ditch Fuhne, MNA could prevent the contamination of the surface water and a subsequent transport of the contaminants on the surface. The second possible scenario is the influx of contaminated water from the industrial area to the west of Bitterfeld into the refilled mining lake Goitsche could threaten the water quality of this recreational area.

Apart from the fringes of the contaminated zone, a second relevant location for using NA as a remediation measure may be at interfaces between groundwater and surface water (Figure 3 B), where sudden changes in oxygen concentration and geochemical conditions can greatly enhance the biodegradation potential for the breakdown of many toxicologically relevant contaminants, such as MCB, VC and chlorophenols (Staps et al., 2002). The latter compounds are relatively persistent under anaerobic conditions and would, therefore be expected to accumulate in the aquifer. An efficient degradation at anaerobic–aerobic interfaces would greatly contribute to protecting the quality of surface waters.

To increase the efficiency of biodegradation, additional electron acceptors or substrates may be added to the aquifer (enhanced natural attenuation: ENA). The microbial population in southeastern part of the Quaternary aquifer at the Bitterfeld megasite has been thoroughly investigated and their potential for the breakdown of the relevant contaminants (like MCB) demonstrated (Wünsche et al., 2000a; Vogt et al., 2002). Efficient biodegradation is limited by a lack of oxygen (e.g. for aerobic degradation of MCB and VC), nitrates (as electron acceptor for anaerobic reactions) and dissolved organic matter (as electron donor for reductive dechlorination) in the aquifer at the Bitterfeld megasite (e.g. Dermietzel and Vieth, 2002; Wünsche et al., 2000b). Hence, providing with these and similar compounds, which may be realized within the construction of reactive barriers or walls described earlier, may increase microbial breakdown rates in the aquifer at relevant locations within the megasite to transfer step by step active, on-site remediation measures like pump-and-treat into intrinsic biodegradation processes.

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