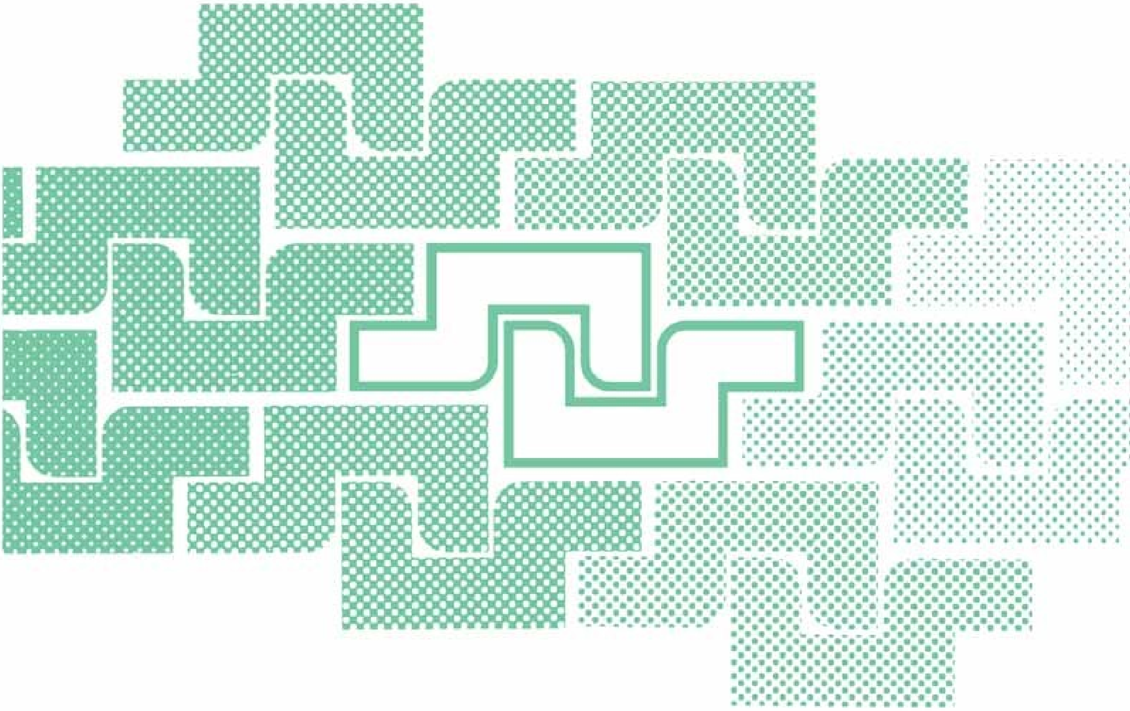

Assessment and Remediation of Contaminated Sediments

Edited by

Danny Reible and
Tomas Lanczos

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IV. Earth and Environmental Sciences – Vol. 73

Assessment and Remediation of Contaminated Sediments

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Assessment and Remediation of Contaminated Sediments

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INTRODUCTION

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On May 18-21, 2005, 56 scientists and engineers convened for an Advanced Research Workshop (ARW) sponsored by the North Atlantic Treaty Organization (NATO). The focus of the ARW was to define and evaluate progress in our ability to assess and remediate contaminated sediments. Contaminated sediments pose some of the most difficult site remediation issues today. They are typically the ultimate repository for contaminants in the environment as a result of runoff and deposition. As such they pose long-term sources of contaminants back to the environment after land contamination issues are identified and controlled. The environmental security of both NATO and partner countries is at risk due to the pervasive nature of sediment contamination of rivers, lakes and harbors.

Contaminated sediments typically reside in spatially variable and dynamic systems subject to seasonal flow variations and episodic storm events. The volume of sediments that must be managed at particular sites often exceeds one million cubic meters, dwarfing many contaminated soil sites. These sediments are also associated with equally daunting volumes of water and efforts to remove the contamination typically entrains even more water. The risks associated with these sediments depend on the processes controlling both contaminant release from the sediments and the transfer to benthic, aquatic, and land-based organisms. Observations of impairments in ecological or human health can indicate potential pollution problems; however, linking these adverse effects to contaminated sediments requires an understanding of the processes leading to exposure and uptake. In addition, the selection of cost-effective and environmentally protective remedial alternatives is dependent upon the ability to predict the risks during implementation and into the future. Due to the volume and complexity of contaminated sediment sites, few economic and effective solutions are available.

Many of the potential technologies for contaminated sediment management were initially developed to manage contaminated soils. Unfortunately, many of these technologies are difficult either to apply or

D. Reible and T. Lanczos (eds.),
Assessment and Remediation of Contaminated Sediments, 1–4.

impose potentially unacceptable risks when applied to contaminated sediments. Identifying, comparing, and selecting remedial options for contaminated sediment is complicated also by the multiple technologies often involved. For example, ex situ treatment or sediment disposal typically introduces a complete train of technologies, including removing material by dredging, temporarily storing or pretreating to reduce water content or volume, treating or disposing of final dredged material, and managing any residually contaminated materials. Large contaminated sediment sites generally require applying different options at different areas on-site, each containing multiple technologies. Therefore, identifying sediment management and remediation options must recognize the entire train of technologies that constitute each option and the interaction of these technologies with the natural sediment processes leading to exposure and risk. Risk reduction has been generally accepted as the metric by which various options are judged and selected. Use of this metric, however, places a premium on understanding the natural processes that link sediment contaminants to exposure and risk. Evaluating management or remedial options requires defining remedial action goals and objectives and developing a valid conceptual model of the sediment system to be remediated, an exceedingly difficult proposition in complex contaminated sediment sites.

The Advanced Research Workshop was directed toward identifying the current state of our understanding of these challenges and progress toward their solution. Many of the problems with managing contaminated sediments have been recognized only in past few years as large contaminated sediments sites such as the Hudson River in the United States have come under scrutiny. Our knowledge and understanding is relatively weak compared to other contaminated site problems. The goal of the Workshop was to ensure that the developing understanding of sediment processes on both sides of the Atlantic are shared and fully incorporated into the thinking of sediment scientists and the regulatory and management community that depend upon their guidance. In the United States, the US Army Corps of Engineers, the US Environmental Protection Agency and University consortia such as the Hazardous Substance Research Center/South and Southwest have focused attention and resources on improving the understanding of contaminated sediment processes and their management. In Europe, a loose organization of scientists and engineers, SEDNET, has led the way in organizing contaminated sediment information. Although there has been interaction among the various groups, the Advanced Research Workshop aided the exchange of information and, in addition, encouraged interactions with Eastern European and partner countries that have heretofore have not focused as much attention or resources on managing contaminated sediments.

The Workshop was organized along the general lines of

1. Identifying the key contaminated sediment issues and approaches in US and Europe
2. Identifying the state of the art in physical, chemical and biological assessment of sediments
3. Identifying the state of the art in our understanding of key fate and transport processes in sediments for both organic and inorganic contaminants
4. Identifying the state of the art in both ex-situ and in-situ management of contaminated sediments
5. Integrating the information exchanged during the workshop to define research gaps

The introductory topic ensured that the participants had achieved some agreement as to the key sediment issues and concerns limiting their management. This was followed by a discussion of the tools available for sediment site characterization and analysis. The translation of these into a valid conceptual model of the system depends upon our understanding of the fate and transport processes influencing contaminants and the resulting exposure to human health and the environment. The Workshop then sought to identify the options available and the key technical issues remaining that limit the ability to effectively manage contaminated sediments. Finally, the Workshop sought to integrate the information to help focus attention on the technical deficiencies in both assessment and remediation that limit our ability to effectively respond to contaminated sediment problems.

It was recognized by those in attendance that in-situ management approaches to contaminated sediments hold significant advantages in complexity, cost and environmental consequences over ex-situ management approaches. As such, the workshop focused much of the discussion on in-situ assessment and remediation. Among the technologies that exhibit the greatest potential for management of contaminated sediments is biological transformation and degradation processes and a significant component of the workshop was devoted to their discussion. This is reflected in this book that summarizes the proceedings of the workshop. In addition, the uncertainties and research needs for realizing the potential of biotransformation and biodegradation processes were also highlighted.

It is hoped that the current volume will provide a window into the discussions held during the ARW for those that were unable to attend and will benefit professionals seeking to gain an understanding of the key issues limiting our ability to manage contaminated sediments. It is toward that goal that this volume is dedicated.

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APPROACHES AND FRAMEWORKS FOR MANAGING CONTAMINATED SEDIMENTS - A EUROPEAN PERSPECTIVE

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Abstract- Sediment is an essential, integral and dynamic part of the hydrologic system. In natural and agricultural basins, sediment is derived from the weathering and erosion of minerals, organic material and soils in upstream areas and from the erosion of river banks and other in-stream sources. As surface-water flow rates decline in lowland areas, transported sediment settles along the river bed and banks by sedimentation. However,

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because sediments are the ultimate reservoir for the numerous potential chemical and biological contaminants that may be contained in effluents originating from urban, agricultural, and industrial lands and recreational activities, contaminated sediments in rivers and streams, lakes, coastal harbors, and estuaries have the potential to pose ecological and human health risks. The management of sediment quality and quantity in support of ecological and socioeconomic goals is a cause of extensive research, investment and regulatory and public scrutiny, both in Europe and internationally. This chapter examines the management of sediment from a European perspective. The first section discusses the role of sediment management in achieving European ecosystem objectives. Section two summarizes the findings of the EC-funded, demand driven European Sediment Research Network (SedNet). Section three describes a framework risk assessment and management in a major European region, the Venice Lagoon. The next three sections describe aspects of sediment management in an EC accession country, and the host country for this workshop - Slovakia. Section four describes river and lake sediment contamination and related legislation in Slovakia. Section five discusses the assessment and management of Persistent Organic Pollutants (POPs), a major risk driver in Slovakia. Section six discusses a specific class of POPs - PCBS in sediments of a specific region of Slovakia. Finally, Section seven discusses some barriers to successful sediment risk assessment and management, and lists science and infrastructure needs to address these barriers. Whilst all the authors of this chapter are living and/or working in Europe, and the focus is on European perspectives, much work has benefited from international collaborations and we feel that many of the observations and recommendations will have relevance to a broader audience.

Keywords: Sediment management; risk assessment; sediment reclamation; Water Framework Directive; Ecosystem; Basin-scale; SedNet; Venice Lagoon, biodegradation, bioremediation, persistent, pollutants, sediments, PCBs, POPs, Sediments quality, sediment contamination by PCBs, the Zemplinska Sirava Reservoir, Vicinity of Strazske

1. Sediment Management: Its role in achieving European ecosystem objectives

1.1. THE EVOLUTION OF SEDIMENT MANAGEMENT

We have been dredging, and thus managing, sediments for centuries. Dredging technology is arguably one of the triumphs of human ingenuity in our battle to control nature. Notwithstanding some of its current press, it has given us our greatest cities, fuelled trade empires and continues to keep nations from disappearing beneath the sea. However, over time, this process grew more complex. The waste products of our other technological triumphs bound with the sediments that were being dredged Thus, dredged material (DM) slowly evolved from a precious resource to a waste material, and environmental assessment and control became a "burden" that was added onto long-established processes. Over time, dredgers developed containment, treatment and disposal technologies to handle these wastes, and thus clever engineering solutions have been developed and applied. However, costs became higher and options were restricted (Apitz 2004).

Now, the field of sediment management can be divided into two general categories, largely defined by the purpose for which they are being examined. The first, construction or navigational dredging, generally involves the assessment and removal of large volumes of sediment, in support of socioeconomic goals. The second type of sediment management, hotspot or environmental cleanup of contaminated sediments, generally addresses smaller volumes of sediment, though there are notable exceptions. The regulatory frameworks and technical communities that address these two sediment categories are often separate, with little or ineffective interaction. Assumptions, methods and frameworks designed to address one category may be inappropriate for the other (Apitz and Power 2002).

However, because the former category of sediment management was well-developed long before the latter was considered, we have historically addressed contaminated sediment management using a DM management-influenced approach. A decade or more ago, removal and treatment of contaminated sediments appeared to be the remedy of choice, regardless of any potential risks. This approach is a given for DM management, but one can call this a "Chemical engineering" approach to management. The consequence was that projected costs for managing contaminated sediments grew to the scale of national budgets. As a result, it is most likely that large volumes of contaminated sediments will be managed in place, and that source control is emerging as a major component of risk management. It can be argued that the goal of sustainable sediment management should be the selection of the least invasive, but sufficiently protective, management strategy. However, the way we assess and manage sediments in support of such an approach is different than if we are assessing the impact of DM disposal (Apitz 2004).

An emerging recognition of this issue has led, in the last decade, to what could be called the first revolution in scale for sediment assessment and management. Instead of treating sediments as volumes of material to be dumped, contained or treated, we now need to look at the micro- and meso-scale interactions. Scientists and decision makers began to consider how contaminants interact with sediments, how contaminants may move between sediments, water and biota and how contaminants might move over time. It is now clear that sediments can bind contaminants in different ways, depending upon sediment characteristics, geochemical conditions and even degree of aging. This can affect contaminant mobility, bioavailability, degradability, fate and risk. An examination of these issues resulted in the important consideration of site-specific Conceptual Site Models to evaluate these processes (Apitz et al. 2005c). Furthermore, it is clear that an understanding of pathways of contaminant transport (mode, media and mechanism) will inform CSMs, help put biological observations in context and help design management strategies (Apitz et al. 2005b). In recent years, scientists throughout the world have carried out extensive research examining pathways of contaminant – sediment – water - biota interaction, and determining how they might affect risks in sediments.

1.2. THE ROLE OF SEDIMENTS IN BASIN-SCALE MANAGEMENT

However, contaminants transfer between all environmental media. We cannot manage one medium without taking this into account, nor can we manage connected sites in isolation. To reduce risk, we must assess and manage it holistically and at the basin scale. Sediment is part of the hydrodynamic continuum – actions on a sediment unit can affect other parcels, resulting in conflicting, counterproductive or inefficient management actions if not coordinated, regardless of goals. There is now a growing recognition that sustainable risk management implies assessment at the basin-scale, managed at the site-specific scale(Apitz and White 2003). A basin-scale risk management framework should be comprised of two principal levels of decision making: basin-scale evaluation (risk prioritization of sites for further evaluation and/or management) and an assessment of specific sites for risks and management options (site-specific risk ranking and management). The growing recognition of this issue can be viewed as a second revolution of scale (Apitz 2004).

Management of risk in a river basin demands that sediment risk management should be closely linked with the management of soil, water, and industrial and agricultural policy. Because of the complexity of interactions, conceptual models are required to identify, quantify and communicate the links between these processes and media (Apitz et al. 2005a). An understanding of the particle and contaminant flows and interactions within a river basin should inform basin-scale evaluation. This can be termed a Conceptual Basin Model (CBM). It describes how materials move and interact between sites and media, leads to increased knowledge about the river basin system and serves as an important communication tool between scientists, decision makers and stakeholders.

1.2.1. *The indivisibility of sediment and soil management*

Development of CBMs makes clear that, as well as an integration of various aspects of sediment management, basin-scale management requires a better integration of sediment and soil science and management. Soil can be defined as "…the top layer of the earth's crust. It is formed by mineral particles, organic matter, water, air and living organisms. Soil is an extremely complex, variable and living medium" (http://europa.eu.int/comm/environment/soil/). Sediment, on the other hand, has been defined by SedNet as "suspended or deposited solids, acting as a main component of a matrix which has been or is susceptible to being transported by water" (Brils 2003). The first thing one notices when comparing these definitions is that the above definition of soil does not specifically exclude sediments. A further examination of the SedNet sediment definition reveals that all soils, during the weathering and transport process, have been sediments, and also suggests that they will be sediments again during their lifetime. Clearly, both soils and sediments often have a common origin – the weathering products of rocks and organic material. Generally, if they are deposited (or formed) in a terrestrial setting, they are considered soils, while if deposited in an aquatic setting they are considered sediments. The greatest distinguishing factors are water and time. Although these differences will result in different biogeochemical, ecological and other behavior in soils vs sediments, in many cases, the distinction between these two materials will be subtle, transitory and, arguably, unnecessary.

1.2.2. *Differences between soil and sediment management*

As urbanization and industrialization resulted in the release of contaminants into the environment, both soil and sediment science evolved to assess and manage the impact of these releases on the ecosystem. In terms of the management of contaminated soils and sediments, there are many commonalities, as they behave similarly: both are accumulators (and long-term donators) of persistent pollutants such as heavy metals and lipophilic organic compounds. However, contaminated sediment investigations have features that make them more complex than water evaluations and, to a lesser degree, soil or terrestrial investigations (NRC 2001, Apitz et al. 2005a). The simple fact that sediments lie under water implies that measurement, observation, and mapping of contaminant and ecosystem characteristics are technically challenging and expensive. Sediments integrate contaminant input from multiple sources within a watershed or coastal region, creating difficulties in tracking the potential sources of contamination. This can lead to ubiquitous, regional 'background' levels of anthropogenic contaminants that are difficult to separate from site specific sources (Crommentuijn et al. 2000). For the same reasons, sediments are, more often than soils, contaminated with multiple chemicals (Long et al. 1995), making risk assessment and management decision-making difficult and complex. The hydrodynamics and geochemistry of aquatic ecosystems are also quite different from those of terrestrial ecosystems. While soils and groundwater can often be isolated from receptors during remediation, similar isolation or removal approaches for contaminated sediments are more difficult to implement successfully; sensitive aquatic biota are more likely, and at times unavoidably, directly affected during the implementation of the remedy (USEPA 2002). Because the benthic community in direct contact with sediments is often near the base of the aquatic food chain, ecologically-based quality criteria can be orders of magnitude lower than those at most contaminated land sites. Together, these and other factors often push the limits of equivalent assessment methods and cleanup technologies for sediment and can at times increase costs significantly over what may be needed to address similar contaminant conditions in soils. On the other hand, some disposal and containment approaches, when available, compare favorably with soil cleanup technologies and some groundwater cleanups can be prohibitively expensive, so it is difficult to make sweeping statements about costs, which are always driven by site-specific conditions. While the benefits of ownership and cleanup of contaminated land, which can subsequently be sold or developed (or both) to offset the costs of remediation, are clear, such benefits are less obvious in aquatic ecosystems. Furthermore, although contaminated soils can often be left in place untreated (assuming there are no immediate ecological risks), the economic need for sediment dredging, whether for navigational or construction purposes, often requires that large volumes of sediments must be removed and managed even when there is no easily available or cost-effective disposal site or remedial technology. However, the management of sediments cannot be completely separated from that of soil and water, as these systems are interrelated and linked, hydrodynamically, even though not always in regulatory terms (Apitz and White 2003, Apitz et al. 2005a).

1.2.3. *Similarities between soil and sediment function*

In recent years, there is a growing recognition that soils and sediments have important functions beyond the agricultural, transport and hydrological functions described above. In the development of the European Thematic Strategy for Soil Protection, it was recognized that soil has six main functions, three ecological and three technical, industrial and socioeconomic (Blum 2002). Although less explicitly laid out, the important functions of sediment have been pointed out by SedNet (SedNet 2004) and others as well. Upon examination, it becomes clear that the functions of soil do not differ greatly from the functions of sediment, with the proviso that sediments, unlike soils, are intrinsically linked with aquatic systems. One of the ecological functions of soil is as a substrate for the production of biomass, ensuring food, fodder, renewable energy and raw materials. Especially in shallow waters, sediment plays the same, albeit less obvious, role in aquatic systems, although a large part of many aquatic food chains also have primary producers in surface waters. Soils, sediments, and the organisms which live in them play major roles in the biosphere/atmosphere/land and aquatic biogeochemical balance, including filtering, nutrient regeneration, transformation, oxygen balance, buffering, etc. in freshwater and marine systems. Soil and sediments are biological habitats and form the biggest gene reserve on the globe. The major controlling factor of habitat type and health is soil or sediment type, whether the habitat is a healthy benthic community, eelgrass for fish breeding, meadows, desert, forest or farmland.

There are three main technical, industrial and socio-ecological functions of soil (Blum 2002), and these also are similar to the functions of sediments. Soils provide the physical basis for technical, industrial and socio-economic structures and their development, for instance industry, housing, transport, sports, recreation and the dumping of refuse. Sediments provide a similar substrate for most structures and developments in aquatic systems. As will be discussed later, a balanced soil and sediment supply is necessary to protect infrastructure, and, as sea levels rise and climate changes, our management of this cycle will be a primary line of defense. Soils and sediments are sources of raw materials, geogenic energy and water. Lastly, both soils and sediments are the memory of our geogenic and cultural heritage, forming an essential part of the landscape and concealing paleontological, stratigraphic and archaeological treasures.

In spite of these critical functions, both soil and sediment scientists and managers have had difficulty engaging the interest of the regulatory community and the public. The public considers sediments invisible or, in the case of dredged material, a waste, whilst soils are taken for granted while they are eroded, covered, contaminated and destroyed. Extensive discussions were carried out in SedNet meetings about how to engage stakeholders and regulators so that the role of sediments was recognized (see www.sednet.org for workshop reports). Similarly, discussions of the European Soil Strategy grappled with how to make soil more 'sexy' to the same audiences (EEB 2002). Contributors to both the Soil Strategy and SedNet are generating documents and recommendations for the EC to convince them that the protection of soils and sediments are critical in holistic ecosystem management, but they are doing this largely in isolation from one another. Because soils and sediment management are, in reality, interdependent, these efforts should be combined.

1.3. EMERGING ECOSYSTEM-BASED POLICY

It is not, however, just the different historical evolution of soil and sediment science that has hindered better coordination between these fields. Rather, those who work within the web of environmental law and regulation have also recognized that the funneling of problems within specific programmatic 'stove pipes', defined in terms of a specific medium, (e.g., water, soil, air), system (e.g., rivers, estuaries, marine systems, watersheds), contaminant (e.g., PCBs, pesticides, Hg), or activity (e.g., navigation dredging, waste water discharge, environmental cleanup), has in many cases presented impediments to achieving efficient, integrated solutions to environmental problems (Apitz and Power 2002, Bridges et al. 2005). As our understanding of ecological systems has evolved, it has become increasingly clear that effective and sustainable management strategies must focus on whole catchments and their interconnected media (chemicals, water, soil and sediment), rather than on one site or issue at a time. While conceptual approaches for addressing these disconnects are being developed (Apitz and White 2003, Heise 2005, in press), significant institutional barriers remain. However, the European Union has recently adopted several Directives, Strategies, Recommendations and agreements which will require a move from sectoral-based to more ecosystem-based, holistic environmental management (e.g. Apitz et al. 2005 in press, Borja 2005 in press, Ducroty and Elliott 1997, Elliott et al. 1999, Reader et al. 2001, Apitz et al. 2005b), which should make the integration of soil and sediment science and management (and many other fields) both simpler and more necessary.

The implementation of the Water Framework Directive (COM 2000) is changing the scope of water management from the local scale to basin (watershed or catchment) scale (often trans-boundary). It aims to establish a framework for the protection of ground waters and inland, transitional (i.e. fjords, estuaries, rias and lagoons) and coastal surface waters that prevents habitat deterioration and protects and enhances the status of aquatic ecosystems, as well as the terrestrial ecosystems and wetlands linked to them. There has also been a movement from addressing problems in isolation on land, in freshwaters, in estuaries or the coastal zone, to integrating these zones, and extending the ecosystem approach to whole shelf areas. The Integrated Coastal Zone Management Recommendation (ICZM) calls for the "…combination of instruments designed to facilitate coherence between sectoral policy objectives and coherence between planning and management" and "improved coordination of the actions taken by all the authorities concerned both at sea and on land, in managing the sea-land interaction", via a national stock-taking exercise, followed by the development of national strategies, international cooperation, reporting and review, ultimately leading to EC legislation on Coastal Zone management (COM 2002b). The adoption of the EU Marine Strategy (COM 2002a) and the recent suggestion of the need for an accompanying Marine Framework Directive will take integrated ecosystem management philosophies from the terrestrial and freshwater areas through the estuaries and coasts to the open sea, including to the shelf (200 nautical miles) areas. None of these initiatives deal explicitly with soil, sediment, or their relationships, but successful holistic management will require that these are managed as the interrelated issues that they are.

Enshrined in the European Treaty are the precautionary principle and the principles that pollution should be rectified at source, that the polluter should pay and that priority should be given to preventative action. As can be seen from the above, environmental management in Europe will now be based mainly upon biological and ecological (rather than physico-chemical) elements, with ecosystems at the centre of management decisions, applied to all European water bodies (Borja 2005, in press). An important part of achieving ecosystem management is the principle of integration embedded in the European Treaty which requires all other policy areas to take full and proper consideration of the European Community's environmental objectives when making policy decisions (COM 2001a). Thus, the Strategic Environmental Assessment (SEA) Directive (COM 2001b) was developed to ensure that environmental consequences of certain plans and programs are identified and assessed during their preparation and before their adoption. In contrast to Environmental Impact Assessments (COM 1985), required to determine the consequences to proposed projects, these will look at the impacts of decisions above or below the project level. Many activities have both intended and unintended impacts upon soil and sediment balance and function, as well as on the many systems with which they interact. If SEAs and EIAs are to properly address these issues, soil and sediment scientists must work together to provide better science and models in support of these goals.

1.3.1. *Implications of emerging policy*

Notwithstanding the ambitions or expectations of the WFD and related policy, the impacts of centuries of human activities will not be eradicated (e.g., Malakoff 1997; Jackson et al. 2001), and the need for continuing sustainable development will remain (Sala et al. 2000). Thus, there is clearly a need for the development of ecological measures and technology that can evaluate the environmental status of aquatic ecosystems, and the potential impacts of both proposed developments and measures carried out to mitigate the impacts of past and projected activities (see, for example, Diaz et al. 2004). To be effective, however, new initiatives and technologies will need to apply realistic conceptual models that are applicable, irrespective of habitat status, and sufficiently sensitive to detect deleterious ecosystem change at a functional level (Diaz et al. 2004).

Although the value of aquatic ecosystems and the services that they supply is considerable (e.g., Costanza et al. 1997; Chee 2004), it is likely that, in many cases, considerations of overriding benefit and disproportionate cost will result in the licensing of activities and developments, in spite of their potential to damage the ecological status of the water body. In such cases, the impact of habitat loss may be minimized via either mitigation (the act of making impacts less severe) or compensation (the act of compensating for economic, resource, or ecological loss via a payment or, in the latter cases, improvement, elsewhere). However, whether due to mitigation or compensation, habitats in different times, locations, and/or conditions will differ in nature and function from those that existed in the past. By creating, restoring, or re-creating habitats, the overall aim should be sustainable and long-term preservation of ecosystem integrity. Elliott and Cutts (2004) suggest that this implies the maintenance or improvement of an ecosystem’s function or carrying capacity. Sorensen et al. (2004) suggest the creation of new habitat, which they term compensatory restoration, can result in a net increase of ecological services; i.e., the processes and goods provided by ecosystems and their component species that ultimately affect human well being.

By adopting this definition, however, the concept of the ecosystem is broadened. In addition to the direct provision of goods such as food, drinking water, and pharmaceuticals, ecosystems also provide the purification of air, sediments, and water; breeding grounds for local and migratory species; biological control of pest species; stabilization of climate; mitigation of flood and drought; and many cultural values, such as recreational and inspirational values. While most ecologists individually have a good idea of what ecological services and carrying capacity are, the science in support of their definition and measurement is often limited (e.g., Covich et al. 2004), dependent on current knowledge and trends in their field of research (Raffaelli et al. 2003), and thus must be refined within a wider context if this concept is to inform management decisions (Elliott and Lawrence 1998; Elliott and Cutts 2004).

Population growth and global change will increasingly impact marine and terrestrial ecosystems in undetermined ways. What is certain, however, is that, as a significant proportion of the human population within Europe live adjacent to waterways, estuaries, and coastal areas, aquatic systems as a whole are particularly vulnerable to disturbance.

Thus, European management is starting to concentrate less on pollution per se and more on the combined affects of multiple stressors at the ecosystem level with a view to ensuring their protection, restoration, and long-term viability. While chemical pollution is increasingly controlled in industrialized Europe, issues of habitat loss are increasing in importance. Elliott and Cutts (2004) propose that much of this habitat loss can be viewed in terms of physical pollution, analogous to the chemical and biological pollution that is more commonly addressed. However, whereas chemical pollution is (arguably) soluble, given a developed technology and finance, and there are more or less standard methods of assessment (e.g., Apitz et al. 2005a, 2005b), less is known about how to correctly assess and manage the impacts of habitat loss and other disturbances on the integrity and carrying capacity of the system (Elliott and Cutts 2004).

Addressing risk at the basin and ecosystem scale, as demanded by the WFD and related directives, requires an understanding of economic, ecological, hydrological, and other processes across many spatial and temporal scales. However, at present, there is a poor understanding, for example, of how aquatic ecosystems function to accurately assess ecosystem health on a site-specific basis (Germano 2001). Adding several layers of complexity and scale will further complicate this problem. Ecological assessment needs to move away from applying simple statistical methods (see, for example, critique in Diaz et al. 2004) that do not allow for full analysis of complex systems toward an approach more analogous to that of medical diagnostics (Germano 2001; Elliott and Cutts 2004; Galloway et al. 2004b). Implicit in the drive toward holistic assessment is the concept that, to have healthy ecosystems, the constituent biota must, in the main, be healthy. This ideal has been greatly aided by recent rapid advances in diagnostic molecular technologies that make it possible to conduct health assessments of individual organisms in much the same way that we evaluate human health (Depledge and Galloway 2005).

It has been stated that "the identification of minor changes due to anthropogenic activities against the background of large scale natural changes will remain difficult, and at the opposite end, the quality of greatly modified areas also will be difficult to assess" (Read et al. 2001). Ecosystem management requires the assessment and definition of baseline conditions so that we can distinguish between the effects of natural and man-made factors (Lewis 1999; DEFRA 2004), and properly assess liability and sources of stresses. Furthermore, as the science to establish links between morphological change and ecology is weak, much more work is required to establish causality (Freeman 2005). The EU FP6-funded Coastal Ocean Benthic Observatories (http://www.cobo.org.uk) program integrates in situ observational and experimental systems to monitor marine benthic habitats in order to understand how anthropogenic impacts affect benthic ecosystem functioning in support, among other things, of the development of biogeochemical measures to assist in the characterization of ecological function, status, and potential in coastal benthic ecosystems. There is a need for similar experimental systems in various aquatic habitats to provide better scientific understanding of mechanisms linking anthropogenic activities and the biological communities that may be the focus of protection, at various spatial, temporal, trophic, and organizational scales (Levin 1992; Apitz et al. 2005b).

For successful, but pragmatic, environmental management to be achieved, 6 tenets must be fulfilled: actions should be environmentally sustainable, economically viable, technologically feasible, legislatively permissible, administratively achievable, and last, socially desirable and/or tolerable (Elliott and Cutts 2004). In the present climate, a 7th tenet may be added—that the actions will be politically expedient. Examination of these 7 tenets reveal that the focus of these requirements is on societal perceptions, wishes, and needs. Thus, while this article has identified the scientific and technical needs necessary to support basin-scale ecosystem management, the success of this more holistic approach to environmental management will depend greatly on how effectively scientists, regulators, stakeholders, and society in general communicate. Thus, a final research need is the development of decision and communication tools that link this complex science to the needs of society.

1.4. CONCLUSIONS

The future should lead towards a better integrated environmental policy, directly linked to the knowledge of 'environmental interfaces' (including soil-sediment, but not only) and how this knowledge could be used to ensure an efficient environmental protection policy. Successful implementation of ecosystem management and strategic assessment will require integration to an unprecedented degree: integration of environmental objectives from the catchment to the coast and, ultimately, to European seas; of the various water and land uses, functions and values; of different skills and disciplines; of previous and emerging legislation and policy into common and coherent frameworks; of technical, socioeconomic and legislative instruments; of stakeholders in decision-making; of the different decision making levels, affecting ecosystem and water status and management among the Member States (Borja 2005 in press, Apitz et al. 2005b). This integration will require extensive collaboration and research to adapt current systems of environmental assessment and management to the basin and ecosystem level.

Clearly, we have been, and continue to, reduce contaminant inputs into the system. However, deposited sediments reflect the history of past and present point and diffuse pollution. Point and diffuse sources continue to enter the system from ongoing agricultural and industrial practices, catastrophic spills and accidents and changes in erosional and depositional patterns due to climate change and anthropogenic activities. These are not transient problems that will soon cease to clutter “normal” sediment management. Rather, we need to re-think the links between our goals and activities, over time.

Risk management thus also requires a careful consideration in another scale – time. Contamination (and thus risk) can result from many types of sources. Unless all these risk sources are managed, risks will continue and spread. We must, then, project risk over time under various scenarios (using CBMs). We must consider the implications of these trends on our management strategies, but also on our criteria and standards (Apitz 2004).

Where are we that we weren’t ten years ago? We have come very far. It is generally accepted that we can only manage risks in sediments if we understand the dynamics of contaminant behavior. Extensive research has supported these goals. It is beginning to be accepted that sediment quality and quantity issues cannot be addressed in isolation. There are many types of risk, and sites and media interact. It is now also generally accepted that we cannot sustainably manage sediments site-by-site but must manage risk in basins. We have begun to develop conceptual approaches to this problem. National and international networks have been established to address these issues.

However, there are still significant barriers to sediment management in the manner described above. While we have conceptual approaches to basin-scale management we still lack joined-up policies, uniform datasets and modeling tools. There is a need to stop separating dredging and cleanup, sediments, soils and waters in environmental management, but the current practice of "regulatory stovepiping" (Bridges and Apitz 2005) creates considerable barriers to progress. Clearly, agricultural and industrial policy, global change, and changing priorities will all affect whether we can achieve our environmental and economic goals. The there is a need for a more joined-up approach to sediment management. We must move from incremental change in sediment management strategies ("evolution"), to a "revolution" in which we understand how particles and contaminants move from the micro- to the macro-scale, from source to sink, historically and into the future, in order to sustainably manage risk to both the environment and our socioeconomic goals (Apitz et al. 2005d).

2. The SedNet perspective on contaminated sediment management

2.1. SEDNET

The European Sediment Research Network - SedNet - was commissioned by the European Commission Directorate General Research (contract No. EVK1-CT-2001-20002) in order to (main objective) set up a thematic network, initially aimed at the assessment of fate and impact of contaminants in sediment and dredged material and aimed at sustainable solutions for their management and treatment. Hence, between 2002 and 2005 scientific, policy and regulatory aspects of contaminated sediments and dredged material were addressed in 17 workshops and 3 conferences organized by SedNet. Europe's leading scientists and major sediment managers contributed to these SedNet activities. The results are summarized in this section, which is extracted from the SedNet booklet "Contaminated Sediments in European River Basins" (Salomons and Brils, 2004). The comprehensive results can be found at the SedNet website (www.SedNet.org) and will be published in 2006 by Elsevier as a series of four books.

Since March 2005, SedNet, through its founding members (see website) succeeded to continue as a self-supporting European Sediment Network. SedNet continues a European network for environmentally, socially and economically viable practices of sediment management on river basin scales. SedNet wants to be a network of sediment professionals (multi-stakeholders & scientists) and to be the independent platform to expert-advice on any issues related to (sustainable) sediment management challenges, positioned between science/knowledge providers and end-users/stakeholders. The SedNet focus is on sediment quality and quantity issues on a river basin scale, including: estuarine/marine sediments, the origin of sediment (soil erosion) and re-erosion of (contaminated) sediments.

2.2. SEDIMENTS AND ITS VALUE

2.2.1. *Sediment*

Sediment is an essential, integral and dynamic part of our river basins. In natural and agricultural basins, sediment is derived from the weathering and erosion of minerals, organic material and soils in upstream areas and from the erosion of river banks and other in-stream sources. As surface-water flow rates decline in lowland areas, transported sediment settles along the river bed and banks by sedimentation. This also occurs on floodplains during flooding, and in reservoirs and lakes. Often the natural sedimentation areas are severely restricted, e.g. because of embankments and the loss of flooding areas as a result of these embankments. At the end of most rivers, the majority of the remaining sediment is deposited within the estuary and in the coastal zone. Natural river hydrodynamics maintain a dynamic equilibrium, regulating small variations in water-flow and sedimentation by re-suspension and resettlement. In estuaries, sediment transport occurs both downstream and upstream, mixing fluvial and marine sediment as a result of tidal currents.

2.2.2. *Its value*

Sediment forms a variety of habitats. Many aquatic species live in the sediment. Microbial processes cause regeneration of nutrients and important functioning of nutrient cycles for the whole water body. Sediment dynamics and gradients (wet-dry and fresh-salt) form favorable conditions for a large biodiversity, from the origin of the river to the coastal zone. A healthy river needs sediment as a source of life. Sediment is also a resource for human needs. For millennia, mankind has utilized sediment in river systems as fertile farmland and as a source of construction material (Table 2.1).

Table 2.1. Overview of sediment as a resource (Martin, 2002).

Too much sediment	Too little sediment	Sediment as a resource
Obstruction of channels	Beaches erode	Construction material
Rivers fill and flood	Riverbanks erode	Sand for beaches
Reefs get smothered	Wetlands are lost	Wetland nourishment
Turbidity	River profile degradation	Agricultural soil enrichment

2.3. CONTAMINATED SEDIMENTS IN EUROPEAN RIVER BASINS

2.3.1. *Contamination*

Sediment acts as a potential sink for many hazardous chemicals. Since the industrial revolution, human-made chemicals have been emitted to surface waters. Due to their properties, many of these chemicals stick to sediment. Hence in areas with a long record of sedimentation, sediment cores reflect the history of the pollution in a given river basin. Where water quality is improving, the legacy of the past may still be present in sediments hidden at the bottom of rivers, behind dams, in lakes, estuaries, seas and on the floodplains of many European river basins. These sediments may become a secondary source of pollution when they are eroded (e.g. due to flooding and channel bank erosion) and transported further downstream.

Along the course of the river to the sea, transportation, dilution and redistribution of sediment-associated contaminants occurs. Many relatively small inputs, all complying with emission regulations, accumulate to reach higher levels by the time sediment reaches the river delta. In the estuary, uncontaminated marine sediments are mixed with contaminated fluvial sediments. This natural ‘dilution’ decreases contamination level in a gradient towards the sea over short distances, but does not alter the actual transported quantity of contaminants.

Despite regular sediment quality assessment by member states, a reliable estimation of the overall amount of contaminated sediment in Europe is hard to give. The main reason for this is the absence of uniformity in sampling methods, analytical techniques and applied sediment quality standards or guideline values. This causes a lack of intercomparability. Typically, countries along the same river basin use different methods.

2.3.2. *Adverse effects of contamination*

Contaminants can be degraded or fixed to sediment components, thus decreasing their bioavailability. At a certain level, contaminants in sediment will start to impact the ecological or chemical water quality status and complicate sediment management. In the end, effects may occur such as the decreased abundance of sediment dwelling (benthic) species or a decreased reproduction or health of animals consuming contaminated benthic species. Contaminated sediments remain potential sources of adverse affects on water resources through the release of contaminants to surface waters and groundwater. Furthermore, contamination adversely effects sediment management, as handling of contaminated material, e.g. in the case of dredging, is several times more expensive than handling clean material.

Clean sediment can also have environmental and socio-economic impacts. For instance, turbidity and excessive sedimentation have a physical effect on benthic life, too much sediment in navigation channels requires costly dredging, and sedimentation behind dams decreases the economic lifetime of that dam. Furthermore, dams decrease the supply of sediment needed to support downstream wetlands, estuaries and other ecosystems. SedNet focused on contamination issues, rather than on such sediment quantity issues.

2.3.3. *Assessment of contaminated sediment*

For the assessment of contaminated sediment, there is not one 'best' method available. Each specific management question requires a tailor-made solution. Chemical analysis can be used to determine concentrations of selected hazardous chemicals and then it can be checked if the concentrations exceed pre-defined standards or guideline values. Using bioassays can test the toxic effects of sediment on organisms. Through a field inventory the long-term impact on sediment biota can be investigated. These assessment methods (chemical, bioassay, field) are complementary by giving a unique answer that cannot be given by any of the individual methods by themselves. But each method also has its own unique drawbacks and uncertainties. A simultaneous or tiered application of these three, complementary assessment methods (Figure 2.1) is commonly referred to as the Triad-approach (Chapman 1996).

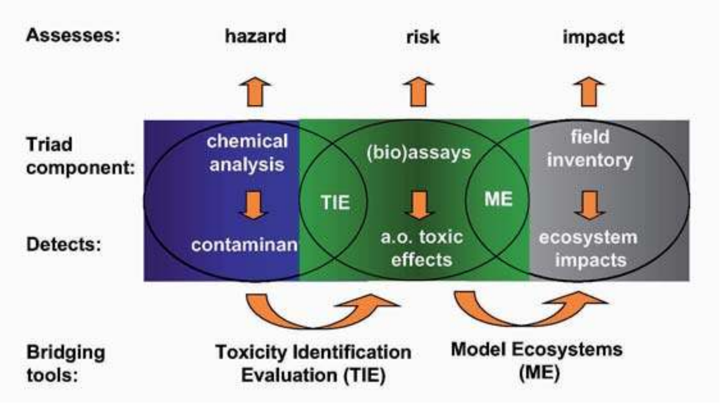

Figure 2.1. An elaboration of the sediment Triad-approach, positioning hazard, risk and impact assessment and positioning the bridging tools Toxicity Identification Evaluation and Model Ecosystems.

2.4. SEDIMENT MANAGEMENT

2.4.1. *Dredged-material management*

Many water and port managers face the continuous effort of dredging in order to maintain the required water depth. Europe-wide, the volume of dredged material is very roughly estimated at 200 million cubic meters per year. There are three types of dredging: capital, maintenance and remediation dredging. Capital dredging is for example for land reclamation, deepening fairways, etc. Maintenance dredging is mainly to keep waterways at a defined depth to ensure safe navigation, and remediation dredging is to solve environmental problems of contaminated sediments. Contamination mainly leads to problems in maintenance dredging because given standards or regulations do not allow the free disposal in the aquatic system. In general with capital dredging old, uncontaminated sediments are being dredged. There is a lot of information on the different options of dredged-material management available.

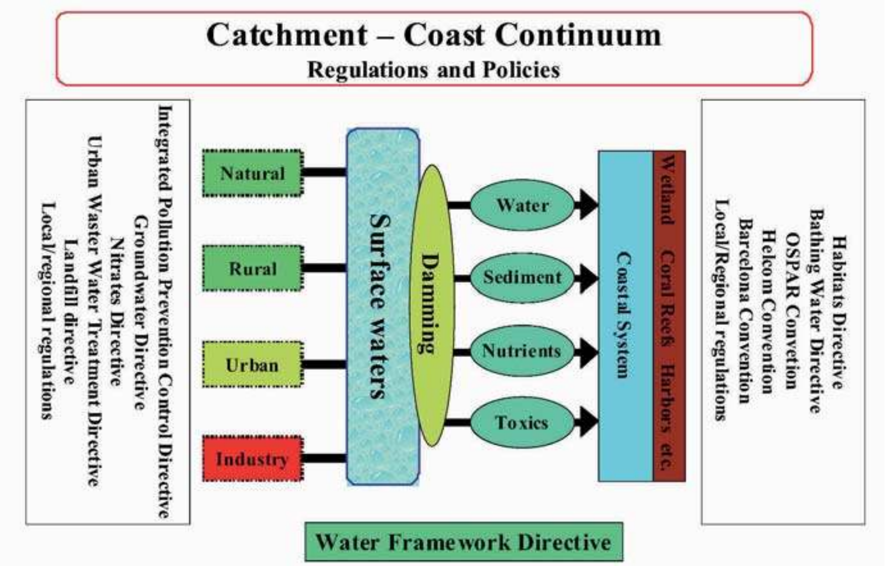

Figure 2.2. The Catchment-Coast continuum and some of the upstream and downstream regulations and policies affecting sediment quantity and quality as well as those relevant for impact assessment downstream.

2.4.2. *Legislation and guidance*

Dredging is mainly done in the coastal or marine environment. Therefore, international guidance has existed for many years to minimize the ecological effects of dredging and open water disposal. European legislation for handling dredged material is complex, because dredged material is at the borderline of water, soil and waste policies. European legislation affects the management of dredged sediments in upland areas, like the waste legislation (Directive 75/442/EEC; Decision 2000/532/EC), especially the EU Landfill Directive (Directive 1999/31/EC), and possibly the Habitats Directive (Directive 92/43/EEC), amongst others. These EU legislations do not (as yet) deal adequately with sediment (Figure 2.2).

The EU Water Framework Directive (WFD)(Directive 2000/60/EC), in force since the year 2000, does not specifically address sediment management. But it can be a tool to tackle the sources of sediment contamination. It offers an opportunity to further improve our knowledge about the relation between sediment quality and water quality and to harmonize quality assessment and sediment management on a river-basin scale.

2.4.3. *Sediment management challenges*

Sediment and dredged-material management challenges and problems relate to *quality* and *quantity* issues. Quality issues relate to contamination, legislation, perception, risk-assessment, source control and destinations of dredged material. Quantity issues mainly relate to erosion, sedimentation, flooding, the effects of damming and the resulting morphological changes downstream. Often quantity and quality aspects are interrelated: the overall umbrella is the river basin.

Quality issues

The management of contaminated sediments in Europe has been mainly the direct concern of authorities dealing with navigable waterways. Contamination can inflict severely the management of dredged sediments. The costs for the removal of excess sediment increases when it is too contaminated for unrestricted relocation. Port managers are concerned that they have to bear the extra costs for managing contamination which is derived from contributions along the river basin. The ‘polluter pays’ principle is far from being applied. The problem is left for the problem owner and there is no link to those that have caused it.

Besides complicating dredging activities per se, contaminated sediment may pose ecological risks or risks to water quality. The relation between sediment quality and risks is complex and site specific, requiring assessment methods based on bioavailable contaminant fractions and bioassays rather than results based on the traditional total contaminant concentrations. However, if sediment quality impairs the chemical or ecological status, remediation measures may be needed. So far, only in a few Member States has contaminated sediment been managed due to its impact on the ecological quality of water bodies.

An integrated approach for sediment management is presently lacking. The WFD aims at source reduction, which in the long term may lead to 'clean' sediment quality. Next to the emissions of point and diffuse sources, a source of increasing importance in this respect is historic contamination, i.e. our legacy of the past. The diffuse sources in which such contamination is present in many European basins are becoming increasingly important. Even more now, since the risk of extreme river floods, that may wash the hidden pollution into the water system once again, seems to have been underestimated in the past.

Quantity issues

Quantity aspects were not a predominant part of SedNet activities. However, they were addressed in several of the workshops since they influence the flux of (contaminated) sediments in river basins. A selection of the issues which were discussed and which have to be taken into account in basin-wide management are:

- the use of sediments in river basins for construction materials with the result of reduced sediment supply downstream, river bed incision and the associated impacts on infrastructure (e.g. bridges etc.);
- changing land use and effects of increased erosion on agricultural soils in particular;
- the effects of damming, reducing sediment supply downstream and resulting in morphological changes to floodplains and deltas;
- damming and the temporal storage of upstream pollutants in deposited sediment (legacy of the past), with the consequence of further transport downstream through erosion events;
- flood control measures, including controlled flooding of areas adjacent to the river, impacting sediment budgets.

Last but not least, climate change and its impacts on the hydrology at the river-basin scale will affect sediment fluxes and should be anticipated in a sediment management plan.

2.4.4. *Management options*

Costly end-of-pipe solutions may be unavoidable for sediment and dredged-material management (Figure 2.3). Solutions like relocation into the aquatic system or placement on river embankments are the first options to consider, since they bring the sediment back to where it belongs. But these solutions are only acceptable if the contamination is below given standards. Depots for contaminated dredged material can be an option in this situation, but they are expensive, often lack public acceptance and are subject to complex legislation. Alternatives include treatment for beneficial use and controlled (confined) disposal. Treatment and re-use is politically encouraged, but is currently applied only at a small scale because of the higher costs compared

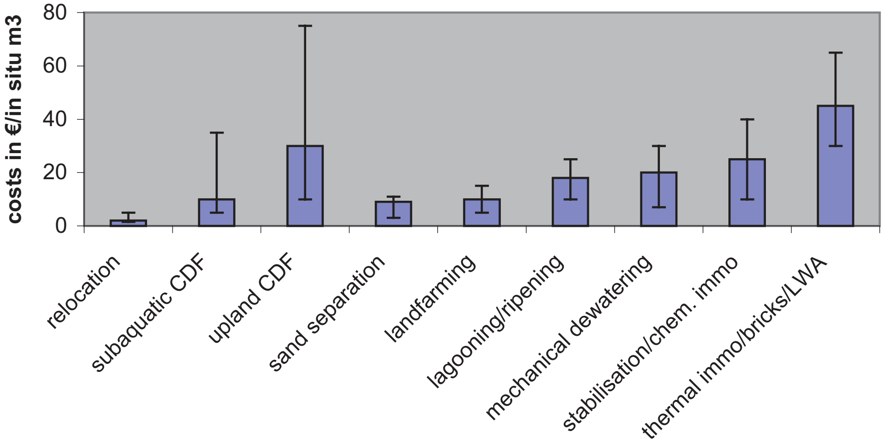

Figure 2.3. Treatment and disposal costs. The height of the columns represent average and the bar shows the range of costs (Netzband *et al*. 2002). Disposal options on the left and to the right treatment options vary in wide ranges due to different boundary conditions. (Note: Revenues or costs for application of the products are not included. The costs for sand-separation do not include dewatering and/or disposal; CDF = Confined Disposal Facility; LWA=Light Weight Aggregate; chem = chemical; immo = immobilization).

to disposal and the lack of product markets. However, in some cases treatment and beneficial use may be a competitive alternative for confined disposal. Confined disposal will remain the first choice solution for the time being. For the realization of new confined disposal sites (both upland and sub-aquatic), public involvement and support are needed.

In many cases the procedures are very time consuming (10-15 years) and/or the lack of public acceptance can complicate matters and their implementation.

2.5. SEDNET RECOMMENDATIONS

2.5.1. *Main recommendations*

As a result of the activities of SedNet, and especially the workshops and conferences, SedNet has developed the following main recommendations:

Towards European policy development:
Further develop and eventually integrate sustainable sediment management into the European Water Framework Directive and related policy and legislation. SedNet viewpoint on sustainable sediment management is further described in the next section.

Towards sediment management:
Find management solutions that carefully balance social, economic and environmental values and are set within the context of the whole river system,

Towards research:
Improve our understanding of the relation between sediment contamination (hazard) and its actual impact to the functioning of ecosystems (ecological status) and develop strategies to assess and manage the risks involved.

2.5.2. *Towards sustainable sediment management*

Sediment issues occur in temporal (geological and seasonal cycles) and spatial scales (catchment area, river foreland, polder) which cross political and administrative boundaries. Yet boundaries tend to scatter sediment management responsibilities and in the end no single stakeholder or country feels responsible. Planning sediment management at the river-basin scale will urge co-operation between agencies and even countries.

At that scale, joint methods and strategies should be developed for sediment and dredged-material management that link to the EU WFD and to pilot projects on trans-boundary rivers. Such methods and strategies should preferably be shared between different basins so that we can learn from each other. It will also help to recognize the differences between basins. This will underline the need to develop tailor-made, realistic solutions towards the environmental and socio-economic management issues that are at stake at that specific basin, or more detailed solutions at specific sites in that basin. For instance the type of dredged material, and the type of contamination, varies considerably between basins and between specific sites within a basin.

Thus sediment and dredged-material management needs to be integrated into existing frameworks at this scale, such as river-basin management plans. An integrated approach is needed from inland (upstream) to coastal waters (downstream). This approach should respect the national and EU policy targets and comply with legislation.

Other recommendations towards sustainable sediment management are:

Find solutions in increase of the interaction with stakeholders. The perception of sediment depends on a variety of roles, values and definitions and is influenced by stakeholder interests. In order to maintain a dialogue, definitions and terms used to describe sediment must be neutral and all-embracing, and sympathetic to stakeholder values and views.

Intervene in such a way that it does not result in unwanted impacts elsewhere in the river basin (up- or downstream), and should not have an adverse impact in the future. A basic understanding of the water system, its dynamics and of the functions of the bordering areas (populations, industries, agriculture) is essential for an effective decision-making process.

Look for integrated solutions that embrace the whole soil-water system. Sediment is part of the soil-water system. Management of sediments should be planned in the context of the soil-groundwater-water-sediment system.

Look for solutions that respect natural processes and functioning. Management strategies for sediment should respect nature: working with nature, not against it. Thus it is crucial to use and improve our understanding of river system functioning and the role of sediment in the processes involved (see further under research recommendations). For instance, taking sediment out of the system can cause sediment deficits resulting in habitat loss and destabilization of river system functioning. Therefore, sediment management must also consider the sediment balance and its dynamic role in the hydrological and geomorphologic processes operating within each river.

3. A framework of risk assessment and management for sediment reclamation in the Venice Lagoon

Persistent and toxic pollutants, as well as nutrients, of both industrial and municipal origin are distributed over the whole Venice lagoon sediment, and heavily contaminated hotspots were identified in the innermost, industrial harbour canals. Taking to account the Water Framework Directive and national/local regulations, the DPSIR framework, proposed by European Environmental Agency, was implemented to support the decision-making and management process. Driving forces, Pressures, and State were investigated through several research investigations and monitoring programs, including: analysis of well-known persistent pollutants as well as new classes of emerging pollutants (*e.g.* endocrine disrupting compounds); development of fate-transport and bioaccumulation models; application of specific bioassays and biomarkers. The research and monitoring results were integrated into an Environmental Risk Assessment to obtain a detailed investigation and evaluation of Impacts. Technological Responses were tested in specific pilot studies including seven individual/combined remedial techniques for heavily contaminated sediment (chemical stabilization by solidification, thermal desorption, solvent extraction, immobilization of heavy metals employing sulfate-reducing bacteria, phytoremediation, metal high gradient magnetic separation and vitrification). Finally, in order to support the decision-making and management process, two GIS based Decision Support Systems (DSS) were defined, integrating the aforementioned tools, methodologies and experimental and technological activities. The GIS based DSS, called

DESYRE, was designed for the rehabilitation of contaminated soils in the industrial area surrounding the Venice lagoon; while a specific GIS based DSS is currently being developed for the monitoring and management of Venice lagoon's environmental quality.

3.1. REGULATORY FRAMEWORK

3.1.1. *European regulatory frameworks of environmental quality*

There are a number of international and national conventions and regulations dealing with sediments, and in most cases with its quality (e.g. for dredging operations) such as London Convention revised in 2000, Oslo-Paris Convention revised in 2004 and Helsinki Convention (1992). Their purpose and primary aim is the environmentally sound disposal (relocation) of dredged material into the sea. Due to national implementation of international convention and EU Directives, the European member countries have developed special dredged material guidelines with different (limited) competences in practice (Sednet, 2004).

The recently adopted Water Framework Directive (WFD) (2000/60/EC) is an attempt to provide a more integrated approach to water management at the river basin level. Although the WFD provides a list with priority substances, sediment fluxes are not explicitly included, even though sediment fluxes have significant impacts on water quality. The WFD requires the adoption of specific measures to progressively reduce discharges, emissions and losses of priority hazardous substances (Decision 2455/2001/CE Annex X). This provision can help tackling existing pollution sources in European river Basins to reduce ongoing sediment contamination.

3.1.2. *National and local regulatory priority substances frameworks for sediment reclamation in Venice Lagoon*

Nowadays, in Italy, there is not a national legislation dedicated to sediment management. The only regulatory references are the decree (D.M. 24/1996) regulating the sea dumping of dredged material and the decree (D.M. 367/2003) implementing the WFD and the following Decision 2455/2001. In the D.M. 367/2003, quality standards are given for 26 priority substances likely to be found in sediment

Being the Venice Lagoon regulated by a special low, Sediment Quality Criteria (SQC) were enforced since 1993. They are based on the levels of contaminants (heavy metals, polycyclic aromatic hydrocarbons, total hydrocarbons and organohalogen compounds). These SQC are comparable with those in use in other European countries and with the criteria used in the USA. Basically, there are three classes (A, B, C) of sediments that can be used inside the lagoon with increasing caution depending on the level of pollutant concentrations: A and B classes can be reused for the restoration of lagoon morphology, C class needs to be disposed of in confined facilities within the lagoon border. Pollutants exhibiting sediment concentrations higher than those of class C (also referred as C^+ concentrations) cannot be reused within the Venice lagoon and must be landfilled after adequate treatment. The amount of sediment to be removed is quite relevant since it is asked by both purposes navigation maintenance and environmental restoration.

Moreover, in order to reduce entry of pollutants into the lagoon, water quality objectives were enacted (D.M. Ronchi-Costa 23/04/1998) together with maximum allowable loads from the catchment basin and treated effluents discharging directly into the lagoon.

3.2. IMPLEMENTATION OF THE DPSIR FRAMEWORK FOR THE VENICE LAGOON'S SEDIMENT MANAGEMENT

The sediment management in the lagoon of Venice was based on the DPSIR scheme (Figure 3.1), proposed by the European Environmental Agency (EEA, 1995) that includes the characterization of Driving forces (i.e. anthropogenic or natural factors triggering the environmental development such as population, industry, or tidal cycles), Pressures (i.e. how a driving force act on the environment: e.g. atmospheric emission, effluent discharge), State (i.e. result of pressures applied to biotic and abiotic resources), Impacts (i.e. alterations resulting from the effects of pressures on the state), and Responses (i.e. human actions on driving forces and/or pressures and/or state to reduce or eliminate the impacts).

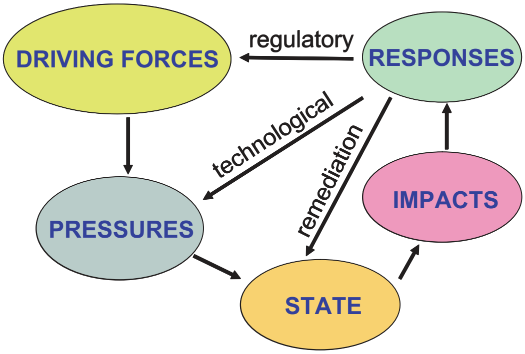

Figure 3.1. DPSIR scheme (EEA, 1995).

Recently, a set of projects and monitoring activities were carried out in the lagoon of Venice, in order to identify the Pressures and to characterize the environmental State. Figure 3.2 shows a schematic summary of the investigations carried out in the lagoon of Venice over the last years.

As far as Pressures are concerned, two large projects were undertaken to quantify the loads of organic and inorganic pollutants, as well as of nutrients, in the lagoon. In particular, the "DRAIN project" (MAV-CVN, 2001) investigated the annual amounts of nutrients (i.e. nitrogen and phosphorus compounds), and organic (i.e. PCBs, PCDD/Fs, HCB, PAHs) and inorganic (i.e. arsenic, cadmium, copper, chromium, lead, mercury, nickel, zinc) chemicals from the catchment area (i.e. river loadings) to the lagoon. The contribution of atmospheric depositions of organic and inorganic pollutants, as well as nutrients, was estimated by the “2023 project” (MAV-CVN, 2000). Moreover, the temporal trends of dioxins (PCDD/Fs) sources were investigated both from experimental data and by applying fugacity models (Dalla Valle et al., 2005a and 2005b).

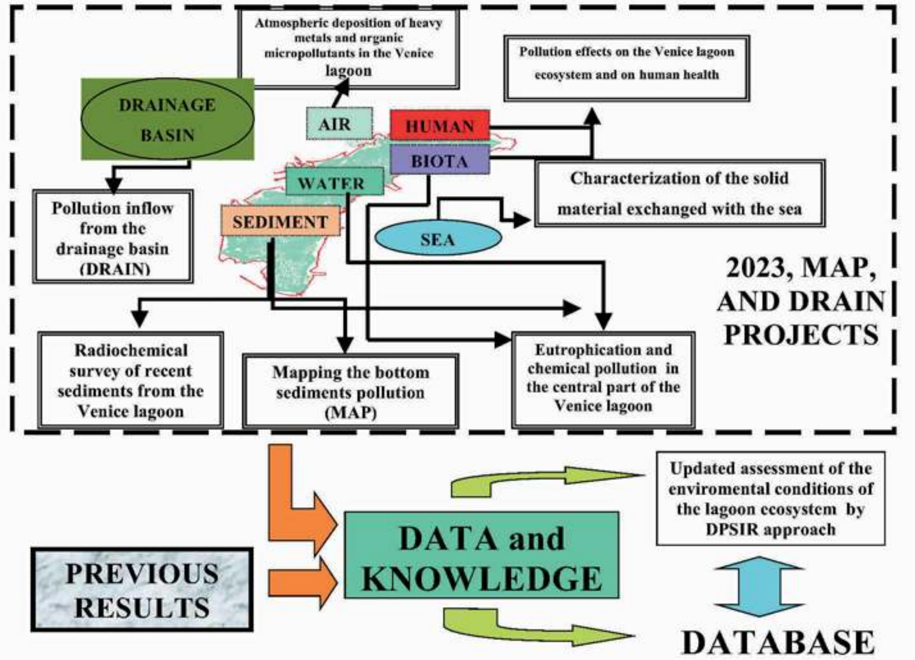

Figure 3.2. Schematic representation of monitoring and research activities carried out in the lagoon of Venice in the last 10 years (MAV-CVN, 2000).

As far the State of the lagoon is concerned, the spatial distribution of organic and inorganic pollutants in the surface sediment and in aquatic organisms was studied by the “MAP project” (MAV-CVN, 1999). Others projects investigated the distribution of PCDD/Fs between environmental compartments (e.g. atmosphere, sediment, water and organisms) (Dalla Valle et al., 2003). Emerging pollutants such as endocrine disrupting chemicals (EDC), especially estrogenic compounds, and pharmaceutical residues, were measured for the first time in sediments, water and organisms (Pojana et al., 2004). Finally, the water concentration of organic and inorganic pollutants over the whole lagoon were monitored within the MELA project (MAV-CVN, 2005).

3.2.1. *Role of environmental risk assessment within the DPSIR framework for the Venice Lagoon*

Based on the Status analysis, the Impacts and Risks for the lagoon of Venice were investigated. In particular, the Ecological Risk Assessment (ERA) procedure (US-EPA, 1998) was applied to identify the chemical stressors of concern and the lagoon areas affected by the higher risk for the ecological resources, with the aim to address the monitoring activities and remediation actions. According to US-EPA procedure, the conceptual model of the Lagoon of Venice (Critto and Marcomini, 2001), defined in the Problem Formulation phase, allowed the selection of three risk hypotheses associated with the accumulation of persistent and toxic pollutants in the lagoon sediments. These three risk hypotheses focused on ecological entities such as the benthic community, the edible clam *Tapes philipinarum*, and the lagoon's food web (Critto and Marcomini, 2001). In order to characterize the potential risk to the benthic community, a screening ERA based on the quotient method (Jones et al., 1999) was developed (Critto et al., 2005). The exposure to different classes of pollutants (metals, chlorinated organic compounds and poly-nuclear aromatic hydrocarbons) was investigated in more than 100 sediment stations, distributed across the whole lagoon, and a map of the spatial distribution of the estimated risk for the benthic community was drawn (Figure 3.3).

Then, the ecological risk associated with bioaccumulation of both total and dioxin-like polychloro biphenyls (PCBs) and several inorganic pollutants (i.e. As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) in clam *Tapes philipinarum* was estimated (Micheletti et al., 2004). A GIS-based map of risk was obtained comparing the pollutants bioaccumulation, estimated by regression models, with internal effects concentrations estimated according to the Tissue Screening Concentrations approach (Shephard, 1998). As example, Figure 3.4 shows the risk for clam associated with cadmium bioaccumulation, one of the main chemical of concern for clam. Finally, an Ecological Risk Assessment for the aquatic food web of the Venice lagoon was carried out, estimating risk posed by the bioaccumulation of organochlorine compounds (i.e. PCBs and PCDD/Fs) through the food web (Micheletti et al., 2005): bioaccumulation was estimated by means of a kinetic Food Chain model (Gobas, 1993) applied to a food web model representative for the Venice lagoon (Libralato et al., 2002). Moreover, the risk posed by the observed concentrations of EDCs in water for biota was analyzed and occurrence of a potential risk for the 24% of the water samples collected in the canals of the city of Venice was highlighted (Pojana et al., 2004).

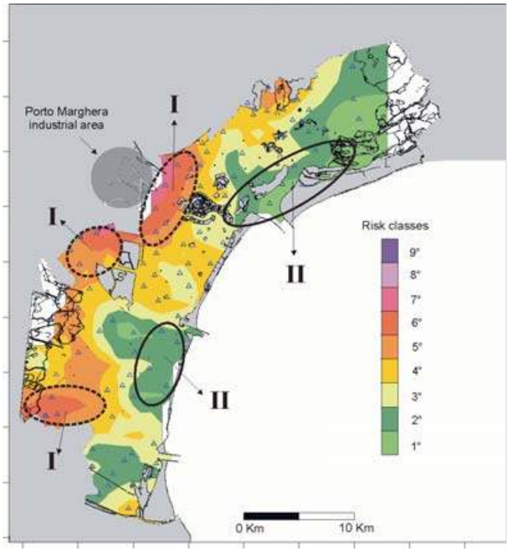

Figure 3.3. Map of risk index spatial distribution for the benthic community (the risk was estimated through the quotient method and the ecotoxicological TEL benchmark). Each risk class represents the number of pollutants exceeding the corresponding TEL benchmark value. The lagoon zones characterized by higher and lower risk class are marked as **I** and **II**, respectively (Critto et al., 2005).

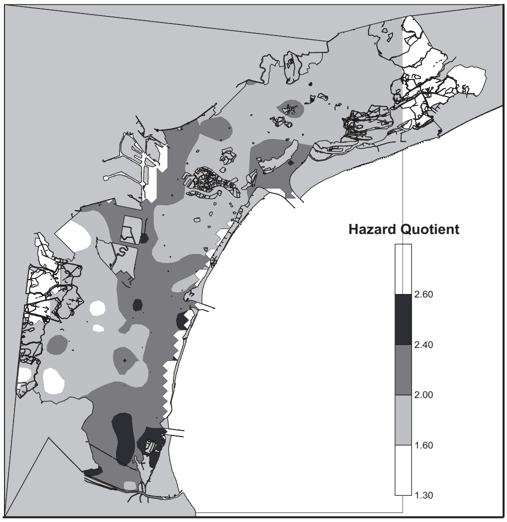

Figure 3.4. Map of the spatial distribution of risk (i.e. Hazard Quotients) posed by cadmium to clam (*Tapes philipinarum*) (Micheletti et al., 2005).

3.2.2. *Responses by sediment remediation technologies: SeRTech project*

With reference to DPSIR scheme, the Responses step (i.e. human actions undertaken on driving forces and/or pressures and/or state to reduce or eliminate the impacts) is a relevant aspect of the sediment management. Recently, the research project SeRTech (Sediment Remediation Technologies), funded by the Italian Ministry of Education and Scientific and Technology Research (MIUR), has been developed by the Venice Research Consortium (CVR) in order to define a technological competitive offer to the reuse/recycling/disposal of contaminated dredged sediment. The main objective of SeRTech project was to select a series of suitable treatment techniques for the most contaminated dredged sediments of Venice industrial canals (over 1.5 million m^3 classified as C^+, see previous section) and to develop an integrated cost-effective treatment system to address the high and heterogeneous contamination compounds (heavy metals, polycyclic aromatic hydrocarbons and organo-cloride) and matrixes, such as those of the Venice lagoon. The re-use and economical potential of treatment by- and final-products has been assessed.

Due to heterogeneous contaminations and matrices of dredged sediments, seven treatment techniques (Figure 3.5) were selected over a large array to perform the laboratory tests (Pippa et al., 2003).

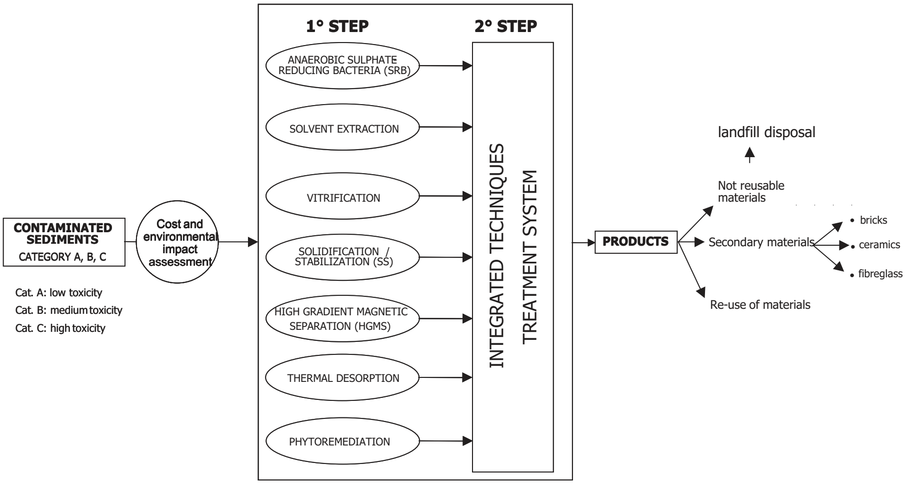

Figure 3.5. Selected remediation processes tested in the SeRTech project.

Two representative macro-samples (4 tons each one), were collected from one of the most polluted industrial canal: they were characterized and used to perform laboratory scale experiments.

Sediments characterization has included: full characterization of pollutants (heavy metals, organic compounds including PCDD/Fs and TBT), physical properties (density, organic matter content, water content, etc), mineralogical analyses, morphological characterization and metal speciation, microbiological characterization, various leaching tests, Microtox test, thermal analysis and other parameters such as nutrients content, salinity, sulfide content.

The assessment of individual treatment techniques (flux and material balance for the most representative contaminants, removal efficiency, energy balance and cost analysis) has led to the definition of an integrated system of technologies (Figure 3.6) to treat both organic and inorganic contaminants, which is based on the material ranking: Low Risk, Medium Risk and High Risk sediment.

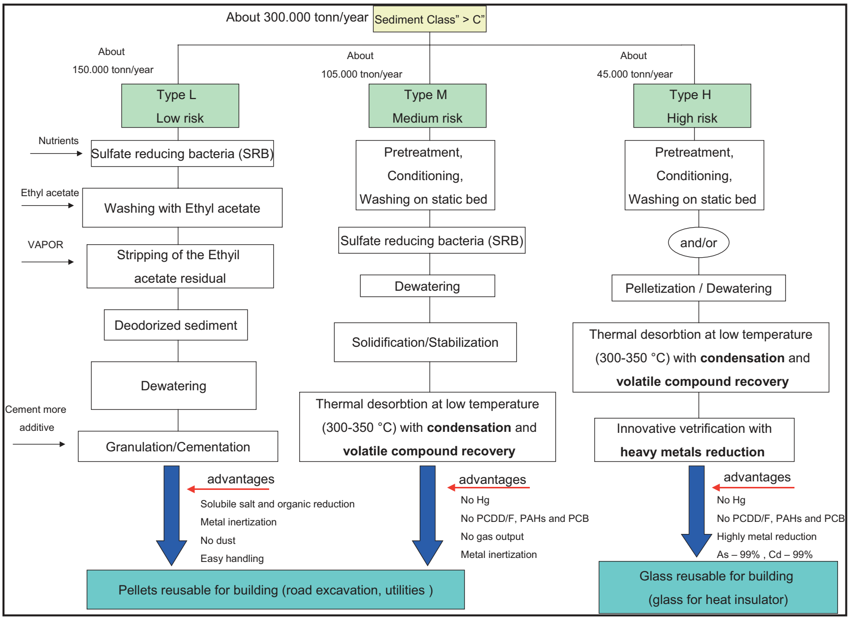

Figure 3.6. The integrated system proposed in the SeRTech project.

The integrated system proposed provides three different technological lines according to different types of contamination level, with a innovative vitrification used as final step only for "High Risk" classified sediment. Different tests were performed to verify this integrated treatment system. The experimentation led to an Italian Patent (patent number VE2004A000050). The final products (glass and pellets) obtained from integrated system were characterized (mechanical properties, leaching tests) in order to verify their reuse in the building industry (i.e. coating materials), roadbeds, etc. A life cycle assessment (LCA) approach was also adopted to compare the different techniques, including environmental benefit and impacts, economical costs and social acceptance criteria.

3.3. DECISION SUPPORT SYSTEMS (DSS) DEVELOPMENT FOR DECISION MAKING AND MANAGEMENT PROCESSES

In the previous paragraphs, tools and methodologies for impacts analysis (i.e. application of environmental risk assessment) and for defining some technological responses (i.e. SeRTech project), have been presented. Nevertheless, more integrated tools are needed in order to assist the definition and planning of responses that deal with the identified pressures and impacts on the ecosystem.

A major current scientific and technical task is to provide the involved authorities with all the relevant information in a manageable format and easy-to-use structure, which integrates technological, economical, social, and political issues and includes sediment management in a more general framework. The development of GIS-based Decision Support Systems, identifying realistic choices and integrating different categories of information into a coherent framework suitable for analysis and selection of alternatives, has proved to be the most effective way (CLARINET, 2002).To this respect, two research projects were developed: one focusing on the definition of environmental quality indices for the Venice Lagoon and one for the definition of site-specific remediation strategies for contaminated sites. The first project was funded by Consortium for Lagoon of Venice Research (CO.RI.LA.), and involves the University of Venice, University of Padua and National Research Council. In order to support the management phase of the proposed DPSIR framework and guide the monitoring process, environmental quality indicators and indices were developed by integrating the information obtained through the application of the DPSIR framework. Exposure and effects data (i.e. lines of evidence) were integrated in the Weight of Evidence and TRIAD approaches, that allowed the formulation of specific indicators/indices of ecological status/integrity.

Within the second research project, funded by the Italian Ministry of Education and Scientific and Technology Research (MIUR), the DESYRE (DEcision Support sYstem for the REhabilitation of contaminated sites) DSS has been developed by the Venice Research Consortium (CVR) in collaboration with the University Ca’ Foscari of Venice, Thetis Spa and CNR-ISE. DESYRE is a GIS-based Decision Support System, which assists the planning and the definition of site-specific remediation strategies for contaminated sites, by integrating environmental, socio-economic and technological information (Carlon et al., 2003). DESYRE, through a user-friendly interface, is addressed to two users groups: one is the multi-disciplinary team of experts (such as risk assessors, socio-economists and technology engineers), who are facilitated in their evaluation work; the other one is the group of several stakeholders (such as public decision makers, planners, site owners, investors and associations), who participate to the evaluation and comparison of alternative remediation options. These options, or scenarios, are described by the system in terms of indices related to technological solutions, risk reduction, cost, time, environmental impacts and socio-economic benefits. As in Figure 3.7, the main interface of the system identifies the subsequent analytical steps. In fact, DESYRE DSS is structured into 6 modules, 5 analytical modules (socio-economic, characterization, pre- and post- risk assessment, technological) and a decision module. The Socio-economic Module addresses the socio-economical constraints through a Fuzzy Logic analysis, until the selection of the best land use (Facchinetti et al., 2003). The site Characterization Module supports the analysis of contaminants spatial distributions by using geo-statistical methods, i.e. variography and Kriging. The Risk Assessment Module is divided into two phases: pre-remediation and post-remediation. In the Pre-remediation phase, an original procedure, based on RBCA methodologies (ASTM, 1998), allows to assess and visualize the spatial distribution of risks posed by contaminants in soil and groundwater, providing a risk-based zoning of the site. The Technological Module allows the definition of the technological solutions and the planning of interventions for the whole site. The system guides the user to the preliminary selection of technologies from a wider database and provides a comparison (through MCDA methodologies) of the selected technologies that support the experts in the definition of several technological sets (Carlon et al., 2004). In the Post-remediation Risk Assessment Module, a simulation of applied technologies provides residual risk maps with related uncertainty maps. Finally, in the Decision Module, alternative remediation scenarios are described by a set of indices. The indices represent the multiple factors derived by the previous analysis: risk, technological, socio-economic, environmental impact, cost and time. The scenarios can be compared and ranked by using Multi-Criteria Decision Analysis methodologies, and presented to the relevant stakeholders in the form of histograms (Figure 3.7).

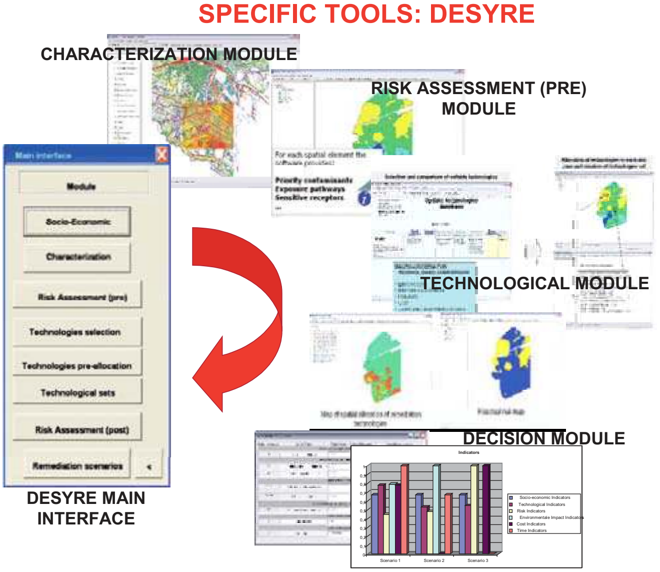

Figure 3.7. DESYRE DSS main deliverables.

DESYRE software was tested successfully in two areas (approximately 450 and 43 ha wide, respectively) of the mega-site of Porto Marghera (Venice, Italy), a large industrial contaminated site of national interest. The application to the different areas verified DESYRE potentialities in dealing with contamination problems at different scales.

3.4. CONCLUSION

In the near future, efforts will be oriented to obtain a Sustainable Management Support System for the lagoon of Venice capable to: integrate Environmental Risk Assessment and Management, improve stakeholders participation to the Risk Assessment process, reduce the uncertainty associated with the management decisions, and to increase the communication between the actors concerned with the lagoon management and the risk assessors. In order to develop such a type of Decision Support System (DSS), environmental quality, socio-economic and technological indices/indicators, need to be identified and quantified.

4. River and lake sediment contamination and related legislation in Slovakia

4.1. INTRODUCTION

Slovakia is a country without water bodies of considerable sizes. The country is without sea coast and big natural lakes, the biggest river in Europe the Danube is touching the southwestern border with Austria and Hungary on the length not bigger than 130 km. Following this information and considering the lack in environmental legislation in connection with sediment contamination, we could easily believe that issues concerning sediment contamination in Slovakia are purposeless. In despite to this Slovakia has a relatively dense smaller river and mountainous stream network because the dissected relief. The total water table area is increased by several dams.

Types of the contaminating chemicals are determined by contaminant sources. Even the anthropogenic pollution sources are very important; we have to take in account the geological background. The geology of the Western Carpathians occupying the bigger part of Slovakia is extremely various what have a very strong influence on the river and lake sediment composition. Regarding anthropogenic sources we may distinguish between industrial sites acting as pollution point sources and extensive agriculture using huge amount of chemicals during the time period between the 50’s and 80’s of the 20th century, acting as area pollution sources. The industry in Slovakia is more-less diverse, but in the second half of the 20th century the dominating potential point sources became huge industrial sites producing chemicals and metal producing and processing enterprises. Maybe the oldest industry in Slovakia was metal ore mining and processing, especially in the area of the Spišské Rudohorie Mountains (see Figure 4.1). Consequently the main sediment contaminating species are different persistent organic pollutants, like chlorinated pesticides and PCBs as well different heavy metals like arsenic, mercury, lead, etc.

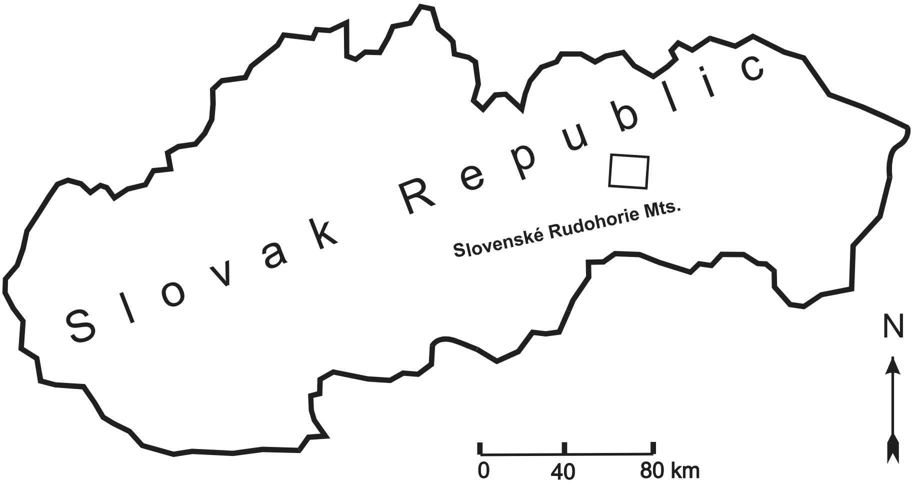

Figure 4.1. position of the Slovenské rudohorie Mts. and the area discussed more in details (see the square).

4.2. SLOVAK LEGISLATION RELATED TO SEDIMENT CONTAMINATION

At the present time neither act nor decree is dealing with sediment contamination issues in Slovakia. For this reason are often used limits adopted from abroad for this reason.

The only document dealing with sediments issued by a governmental institution is the Guideline No. 549/98-2 for the Contaminated River and Lake Sediment Assessment Methods. This document was issued by the Ministry of Environment of the Slovak Republic in August 27th 1998. The goal of this document is to unify risk assessment methods and analyses. Particular procedures in this guideline are forming an integrated system consisting of the following components:

sediment sampling,
chemical analyses of the sediment,
ecotoxicological tests,
biological analyses of the macrofauna living on the water bodie’s bottom,
the highest priority contaminants selection.

The guideline is recommended to apply for:

watercourse and lake sediments pollution degree assessment,

further sediment pollution prevention leading to overreach acceptable environmental and health risks,

inventarisation of the sedimentation areas' contamination degree,

identification of the excessively contaminated areas, namely those which are causing endangering of the water ecosystems and people's health,

monitoring and surveying of sites with contaminated sediments.

This guideline was designed as a complex document dealing with data gathering, sediment sample's processing and analyses, ways and procedures of data processing and interpretation. Within the certain time period the document will be expanded by guidelines for particular scenarios, e.g.:

type of the contaminating species (e.g. heavy metals, pesticides, oil species)

type of the contamination source (point and area contamination sources, atmospheric deposition)

sediment use (recreation, protected areas, fishing, sediment exploitation ..)

endangered target groups (water fauna and flora, predators, people …)

The assessment methods are defined for the national level and for site specific risk assessment. On the ***national*** level the method are based on the concetration estimation in the sediment (***PEClocalsed***) and it's comparison with the ***PNEC*** = Predicted No Effect or with the ***SQC*** = Sediment Quality Criteria for the given species. The output is the relative risk score (***RQ***). The ***Site Specific Risk Assessment*** is based on chemical analyses, ecotoxicological test and the biological quality assessment.

4.3. OVERVIEW OF ARTICLES DEALING WITH POLLUTED SEDIMENTS IN SLOVAKIA

Several studies were published about sediment pollution in Slovakia. Most of them are related to particular case studies, especially related to river dam sediments, e.g. the Palcmanská Maša (Brehuv, 2002) or more case studies about the Šírava dam (see the next article in this book).

Distribution of the contaminants in sediments all over Slovakia is described in regional works, e.g. the distribution of the pesticides was elaborated by Korenková and Slobodník (2001), the relationships between the suficial water and sediments in the Slovak watercourses was evaluated by Hucko (1994).

The most comprehensive description of metal distribution in stream sediments all over Slovakia is provided by the Geochemical Atlas of Slovakia, Part Stream Sediments (Bodiš and Rapant, 1996), published by the Dionýz Štúr Geological Institute in Bratislava. The Atlas was published together with the Geochemical Atlases for groundwater, soil, forest biomass, rocks and natural radioactivity. The Atlas contains maps of distribution for the following elements: Li, Na, K, Rb, Be, Mg, Ca, Sr, Ba, Y, Ti, Zr, V, Cr, Mo, W, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, B, Al, Ga, Tl, Sn, Pb, P, As, Sb, Bi, Se. These elements were analyzed in the stream sediment samples of fraction minus 0.125 mm, mineralogically containing clay, primary silicate minerals, rocks, oxides and hydroxides. The sampling density was 1 sample/2km^2, what means 24 432 samples, sampled during the time period 1991 – 1995.

4.4. CONTAMINATION SOURCES

As I mentioned in the introduction, as important contamination sources we are considering chemical production sites and metal processing and production sites, including metal ore mines.

The rapid changes in the political system and economy in the former Czechoslovakia brought also changes in the industrial production. Typical features of those changes were a shift from the prevailing heavy industrial production to smaller enterprises, the overall production rate dropped also. The pesticide consumption within agricultural production was dropping dramatically due the insolvency of the farmers. Due those circumstances the total influx of contaminants to sediments dramatically decreased, however the accumulated amount of persistent pollutants decreases very slowly.

Polluting species originating from the chemical industrial sites are diverse. The most serious sediment pollution case is the huge PCB pollution of sediments in the Šírava dam in Eastern Slovakia. The pollution source is a huge chemical industrial site owned by the Chemko Company in the city of Strážske. This case is described in details in another contribution of this book.

The metal ore deposits in Slovakia are located in more regions, but the most important region is the Slovenské rudohorie Mountains (Slovak Ore Mts.), especially its easternmost part, also called the Spiškogemerské rudohorie Mts. Following the earliest written evidences the mining activities started in the 12th century (Chalupecký, 1981). Most of the metal ore mines were closed and flooded in the beginning of the 90's, consequently the production of the metal production and processing plants bound to the metal ore mines decreased dramatically and reduced mainly to scrap processing and in some cases to ore processing imported from abroad. Those events also caused decreasing of the heavy metal flux in to sediments. However, if we take in consideration that the mining activities started at least 800 years ago, the sediments contains a huge amount of heavy metals accumulated during this time period.

The character of the mine pollution is strongly bound to the geological background. The deposits are characterized mainly as hydrothermal ore veins, mineralogically built up by siderite, calcite, quartz, baryte and different sulphide minerals, especially by pyrite, chalcopyrite, tetrahedrite, locally occurring also cinnabarite.

4.5. SELECTED HEAVY METAL POLLUTION SOURCES DESCRIPTION

As representatives of typical heavy metal pollution point sources in the Slovenské Rudohorie Mts. three source groups were selected:

- **Rudňany -** iron and mercury ore mine, ore processing plant and sludge deposit ,
- **Slovinky -** copper ore mine, ore processing plant and sludge deposit and the
- **Kovohuty a.s.** copper smelter and related industrial waste deposit in the city of Krompachy.

These sites are located in the northern part of the Slovenské rudohorie Mts. and belong to the upper part of the Hornád river basin.

The Rudňany ore deposit was exploited for several centuries until the beginning of the '90s of the 20th century. The mineral composition of the ore veins is represented by barite, siderite, sulphides (pyrite, chalcopyrite, tetrahedrite, cinnabarite). The deposit was used for iron ore (siderite) exploitation, since the 17th century also copper, silver and mercury. At the end of the 20th century also barite was exploited from the upper part of the deposit (Rapant et al. 2003).

The first written evidence about ore exploitation in the Slovinky ore deposit originates in the 13th century (Chaluopecký, 1981). The deposit is formed again by the typical hydrothermal ore veins containing quartz, siderite with low content of iron, ankerite, calcite and different sulphides: mainly chalcopyrite, pyrite and tetrahedrite. The most important exploited metal was copper gained by chalcopyrite processing, in the past also gold and silver (Rapant et al. 2003). The mining was stopped in 1991.

The processed ore material – copper concentrate was further processed in the Kovohuty copper smelter in the adjacent city of Krompachy, placed on the alluvium of the Hornád river. Although the copper was produced in smaller smelters within the wider area earlier, this smelter started as iron and steelworks in 1835 and finished in 1918 as the biggest iron and steel producing factory in the former Austrian Hungarian Monarchy. In the 20's of the 20th century started with copper producing only and until the beginning of the 90's processed the copper concentrate in Slovinky. In the 60's the production was diversified by manganese, zinc and sulphuric acid production. In present time the smelter is processing copper scrap only (Hyde, 2004a).

The huge production is connected with production of enormous amount of industrial waste. The produced slag in the 19th century was deposited in the adjacent area of the factory. As the factory was growing, the new buildings and plants were built on the slag deposit, placed on the alluvium. In the present time all the industrial area of the city is built on the slag. The production diversification in the 60's was connected with production of different kinds of sludge deposited on the Halňa waste deposit, placed at the easternmost part of the industrial area, also on the alluvium of the Hornád river. The waste deposit was used in the beginning for iron smelting slag deposition, since the 60's until 2000 when the deposition was stopped also for different kinds of industrial sludge and municipal waste (Hyde, 2004a).

4.6. THE ENVIRONMENTAL LOAD AND SEDIMENT POLLUTION CONNECTED WITH THE DESCRIBED SITES

All the described sites are serious heavy metal pollution sources especially for water environment. For the documentation of their impact I used data from the stream sediment sample analyses database created for the purposes of Geochemical Atlas of Slovakia part Stream Sediments (Bodiš and Rapant, 1966). The samples were collected during the time period 1991 – 1992.

The way of the transportation of the metals is various and dependent on local conditions and the character of a particular source. In the past, during the operation of the ore processing plant in Rudňany huge amounts of mercury vapors were emitted to the atmosphere causing visible damage of the surrounding vegetation and contaminating the soil on the adjacent hill slopes. We should take in consideration this way of transportation, but the leachates formed in mine tailings and sludge deposit contains also considerable amounts of mercury. In comparison with the next two sites, the stream sediments around Rudňany contain highest mercury concentration (see Figure 4.2), up to 127.4 $mg.kg^{-1}$.

One of the common features for both of the Rudňany and Slovinky hydrothermal ore deposits is the presence of barite (barium sulphate) in the upper parts of the deposit. However, if we compare Ba concentrations in stream sediments close to Rudňany with stream sediments close to Slovinky, we will observe incomparably higher Ba concentrations in Rudňany, up to 18 010 $mg.kg^{-1}$ (see Figure 4.3).

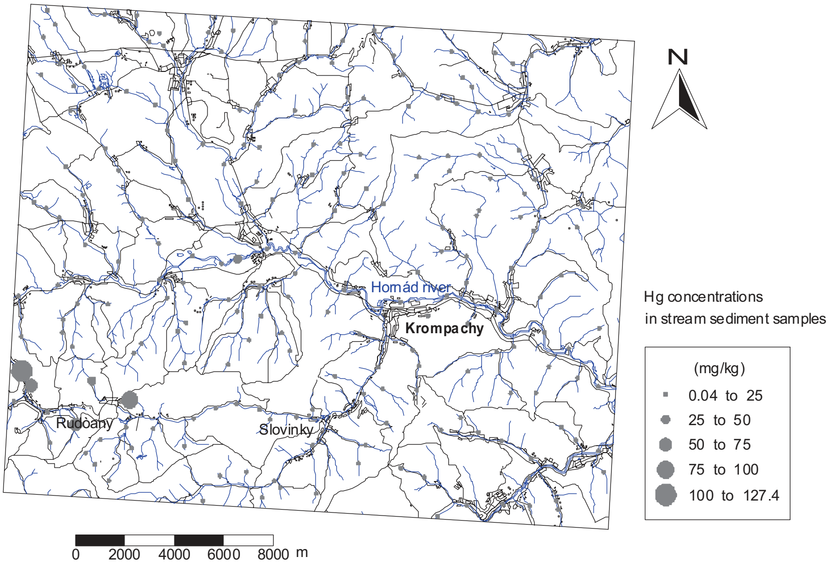

Figure 4.2. Mercury concentrations in stream sediment samples, following data in database created for the purposes of Geochemical Atlas of Slovakia, Part Stream Sediments (Bodiš and Rapant, 1996), gathered from the Geofond archive, Bratislava.

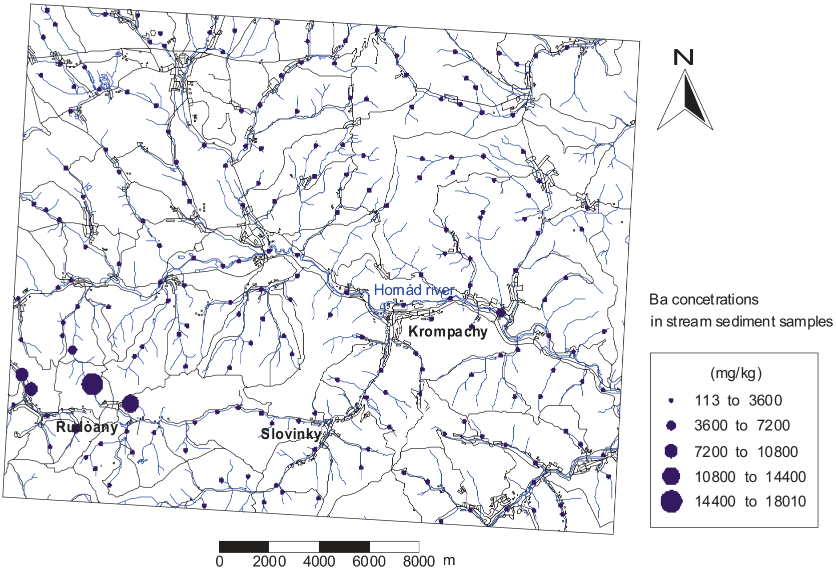

Figure 4.3. Barium concentrations in stream sediment samples, following data in database created for the purposes of Geochemical Atlas of Slovakia, Part Stream Sediments (Bodiš and Rapant, 1996), gathered from the Geofond archive, Bratislava.

The opposite situation is apparent following comparison of copper concentrations. The copper is extracted from chalcopyrite ($CuFeS_2$) occurring in both of the deposits, however the highest concentrations are occurring in stream sediments close to the mine tailings related to the Slovinky ore deposit (up to 2898 mg.kg^{-1}, see Figure 4.4). This difference is again caused by copper utilization in Slovinky. Higher concentrations of copper were observed also in sediments below the Kovohuty copper smelter and belonging Halňa waste deposit.

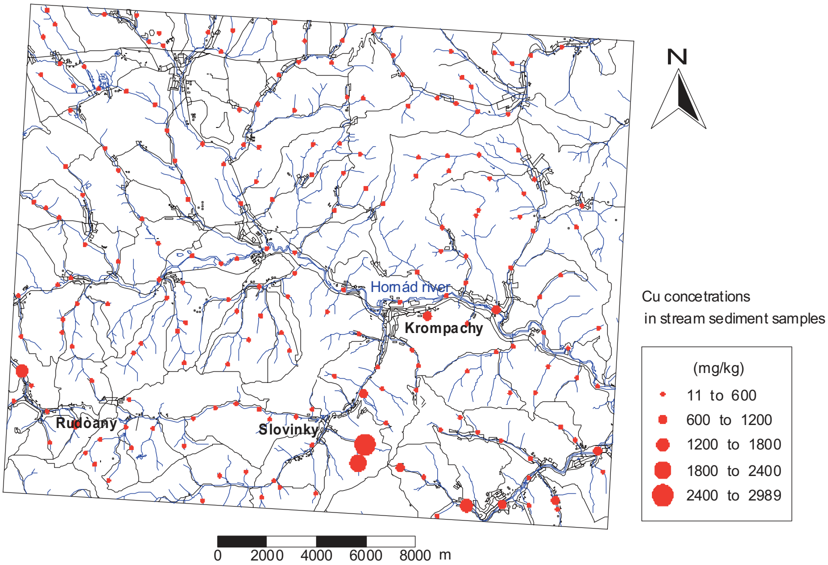

Figure 4.4. Copper concentrations in stream sediment samples, following data in database created for the purposes of Geochemical Atlas of Slovakia, Part Stream Sediments (Bodiš and Rapant, 1996), gathered from the Geofond archive, Bratislava.

Arsenic is often found in sulphidic minerals substituting sulphur in theirs crystal lattice, but the main arsenic containing minerals in the Slovinky ore deposit are arsenopyrite (FeAsS) and tetrahedrite ($(Cu,Fe,Ag,Zn)_{12}Sb_4S_{13}$.) with high As content (verbal information from Mr. Otto Stettner, former chief geologist of the Slovinky ore deposit). Arsenic in water solution is occurring as arsenate (III or IV) anions, what means that is more mobile under more alkaline conditions (Appelo, Postma, 1993). Also in groundwater polluted by sludge deposit leachate of the Halňa waste deposit was reported a correlation between As concentrations and pH values, where the As is the main pollutant (Hyde, 2004b). This is probably the reason of occurring of As polluted sediments in the Slovinsky creek below the sludge deposits and also increased As concentrations in the sediments below the Halňa waste deposit (see Figure 4.5).

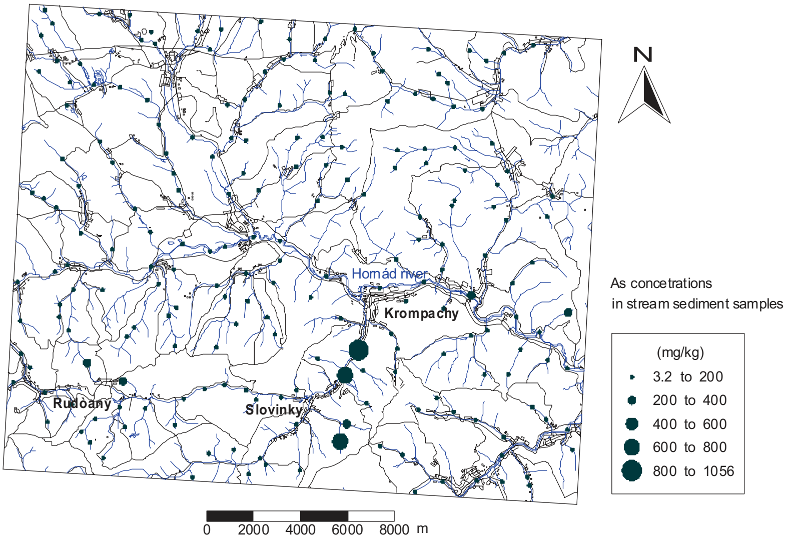

Figure 4.5. Arsenic concentrations in stream sediment samples, following data in database created for the purposes of Geochemical Atlas of Slovakia, Part Stream Sediments (Bodiš and Rapant, 1996), gathered from the Geofond archive, Bratislava.

The tetrahedrite is probably source of antimony in sediments nearby mine tailings of both of the deposits (see Figure 4.6). Antimony is transported by similar processes as arsenic; the different distribution between the two deposits is caused probably by lower As concentration in minerals of the Rudňany waste deposit.

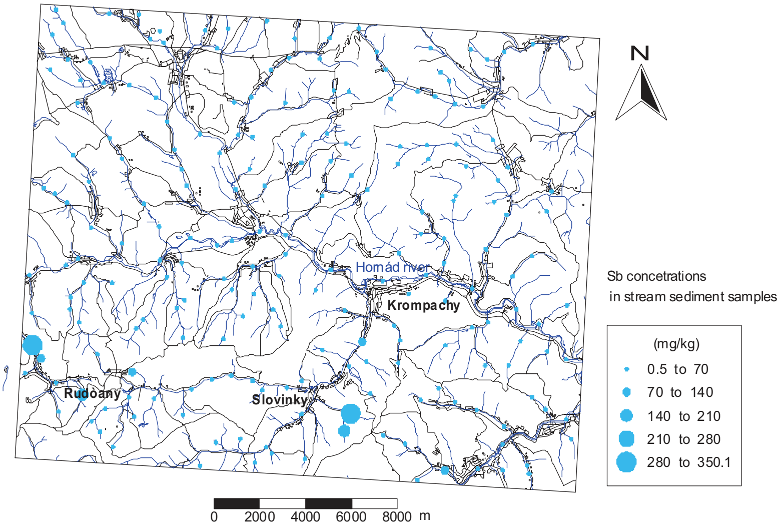

Figure 4.6. Antimony concentrations in stream sediment samples, following data in database created for the purposes of Geochemical Atlas of Slovakia, Part Stream Sediments (Bodiš and Rapant, 1996).

4.7. CONCLUSIONS

This section presented information related to sediment pollution in the region where mining and metal processing activities were running for centuries. My goal was to point on a need for individual approach to different sites; even they are geographically close to each other and on the sites were running similar activities. Those differences are determined by complicated geological background and dissected terrain. Unfortunately, regarding mobilization of heavy metals, their transport in aquatic environment and bond to the sediments, eventually remobilization, in most cases we are only guessing.

The discussed area is mountainous area with fast flowing creeks and small rivers. During storm events and snowmelt periods these watercourses has significantly increased discharge what is causing also moving the sediments downward. Definition of sections were the sediments are transported during these events and sediment transport barriers where the sediments are accumulated should be the another task of investigation.

It is symptomatic, that the serious sediment pollutions in Slovakia presented in this and the next paper dealing with the PCB pollution of the Šírava dam are related to stopped industrial activities. This situation is widespread and raises questions about responsibilities and remediation funding. In the case of heavy metal pollutions caused by mining activities it is more complicated, as these activities were running for several centuries. Moreover funding of remedial projects by different financial mechanisms is mostly bound to clarified landowner relationships what in some situations is unsolvable obstacle. Helping this situation will need some legislative interventions, to define responsibilities and ways of funding investigations and remedial actions.

5. Problems of persistent organic pollutants (POPS) in Slovakia: environmental and technical aspects of PCBs disposal, monitoring and remediation

The National Implementation Plan of the Stockholm Convention commitments and Non-combustion Decontamination Project are discussed. Slovak Republic, a part of former Czechoslovakia, belonged to the eight world's largest producers of PCBs. Safe disposal of PCB wastes from the production, PCB-containing equipment, and environmental parts has to be carried out. The pollution of an open wastewater canal sediments in Strážske locality and a resulting pollution of the Laborec River and Zemplínska Šírava water reservoir sediments constitute major problems. Strategies and action plans at the national level are discussed as well.

5.1. PERSISTENT ORGANIC POLLUTANTS: INTERNATIONAL COMMITMENTS

Persistent organic pollutants (POPs) or persistent bioaccumulative toxic substances (PBT) are carbon-based chemicals that resist degradation in the environment and accumulate in the tissues of living organisms, where they can produce undesirable effects on human health and the environment at certain exposure levels. Concerning such characteristic properties of POPs as *low degradability, low water solubility, high lipid (fat) solubility, bioaccumulation in living organisms, toxicity, semi-volatility, high sorption ability as well as due to migration of POPs at long distances from their original source,* it is necessary to deal with the release of POPs to the environment at multinational level.

Two international conventions are presently adopted, which have the goal to protect human health and environment against the adverse effects of POPs: the *Protocol on POPs to the UN ECE Convention on Long-range Trans-boundary Air Pollution* (1998) and the *UN Stockholm Convention on POPs* (2001). Slovakia is party to both international commitments. The European Union is party to these conventions as well, and in addition adopted also the Community Policy for POPs Management, which upon joining the EU has become mandatory also to Slovak Republic (SR).

Stockholm Convention concerning persistent organic pollutants requires from the signatories to take measures for source elimination of the 12 substances (so called “dirty dozen”) belonging to persistent organic pollutants. The measures include: a) direct production control of POPs pesticides (Aldrin, Dieldrin, DDT, Endrin, HCH, Chlordecon, Mirex, Toxaphene); b) elimination of use of equipment and wastes containing industrially produced PCBs (Delor, Hydelor, Delotherm); c) minimization of unintentionally produced POPs emissions (PCDD/F, HCB, PCBs, PAHs).

5.2. NATIONAL IMPLEMENTATION PLAN: STRATEGIES AND ACTION PLANS

Through Regional Centre of UNDP, Slovakia has obtained a GEF grant to support implementation of the Stockholm Convention commitments and to elaborate the *National Implementation Plan (NIP).* In compliance with the GEF Guidelines, NIP considers all POPs-related international commitments relevant to the SR.

Slovak Republic, formerly Czechoslovakia, is one of the countries where production of the mentioned dangerous substances, specifically PCBs, took place in the past. SR belonged to the eight world largest producers of PCB commercial mixtures (formerly producer Chemko Strážske). Thus, safe disposal of PCBs wastes from the production and

PCB-containing equipment and environmental parts, namely so called "old environmental burdens", has to be done. The Ministry of the Environment of the SR (ME SR) in cooperation with the Slovak Hydrometeorological Institute (SHMU) and other stakeholders were responsible for the project *Initial assistance to the Slovak Republic to meet its obligations under the Stockholm Convention on Persistent Organic Pollutants (POPs).*

NIP contains the following action plans and strategies:

1. POPs Pesticides (Aldrin, Dieldrin, DDT, Endrin, HCH, Chlordecon, Mirex, Toxaphene);
2. Equipment and wastes containing industrially produced PCBs (Delor, Hydelor, and Delotherm);
3. Unintentionally produced POPs (PCDD/F, HCB, PCBs, PAHs);
4. Contaminated sites and releases from stockpiles and wastes;
5. Monitoring;
6. Reporting and information exchange;
7. Raising public awareness of POPs;
8. Institutional and legal measures;
9. Research and development.

5.2.1. *POPs pesticides*

Hexachlorobenzene: was manufactured at a Bratislava chemical plant in the 60´s and 70´s. It was used mainly as a pesticide until its use was banned in 1976.
DDT: was manufactured at a Bratislava chemical plant in the 1960´s and 70´s. Its use in agriculture was banned in 1976, however, in spite of that fact, it was used until the depletion of DDT stockpiles.

Other chlorinated pesticides (Aldrin, Chlordane, Dieldrin, Endrin, Heptachlor, Mirex,_Toxaphene): have neither been produced nor used (perhaps only in small amounts, e.g. Toxaphene) in Slovakia. Their use is currently prohibited. Storage and destruction of obsolete stockpiles remain a problem. An inventory of obsolete POPs pesticide stockpiles in the years 2000 and 2003 documented more than 28 tons of stored POPs pesticide preparations in Slovakia.

5.2.2. *Equipment and wastes containing PCBs*

Altogether, more than 21,000 tons of PCBs were produced in Slovakia during the period 1959-1984, and broadly utilized in former Czechoslovakia mainly for production of capacitors, paints, and varnishes. Basing on extensive inventories, which were carried out in the years 2000 and 2002, current existence of about 3,500 tons of PCBs may be assumed in the territory of Slovakia. Another 900 tons of PCB containing wastes are stored at the landfill near by the factory. It is assumed that the total amount of identified PCB wastes will still increase, after the new waste management legislation will come in force.

5.2.3. *Unintentionally produced POPs*

The release of unintentionally produced POPs in Slovakia had decreasing trends in the period 1990-2001. The PCDD/PCDF emissions decreased by 76 %, the PAHs by 65 %, HCB by 74 %, and PCBs by 85 %. Gradual implementation of environmental measures in the main polluting sectors (metallurgy, waste incineration, energy sector, and transportation) triggered by the introduction and application of the new BAT/BEP based environment protection legislation, were the main reasons for this decrease.

5.2.4. *Contaminated sites and releases from stockpiles and wastes*

The pollution of an open wastewater canal in Strážske locality and resulting pollution of the Laborec River and the Zemplínska Šírava water reservoir, as a consequence of previous PCB production, constitutes a major problem. This contamination resulted in increased values of PCB content in the monitored components of the environment as well as in the population of Michalovce District, comparing with the rest of Slovakia.

Another important group of contaminated areas polluted with PCBs includes those located in the vicinity of asphalt-gravel mixing plants. Their contamination is caused by improper handling of PCB containing heat-exchange fluids.

5.2.5. *Monitoring*

POPs, in particular PCBs, DDT, and HCB, are monitored in an uncoordinated manner in all the components of the environment, living organisms (including humans), and food chain. None of the monitoring programs covers the whole area of Slovakia. The highest POPs levels were detected in the human population and house animals in Michalovce District (Eastern Slovakia). Regarding the components of the environment, the highest POPs concentration levels were detected in soils and in certain localities (Strážske region) also in sediments. In general, decreasing tendency of POPs presence prevails in all monitored matrices.

5.2.6. *Institutional and legal measures*

Legal regulations concerning POPs are intersecting through several government departments (healthcare, environment, agriculture, and finance-customs). The main driving force influencing the formulation of the new legislation is, besides demands of the international treaties, also a necessity to harmonize the Slovak legislation with that of the EU, in particular with the Proposal for Regulation of the European Parliament and of the Council on POPs and amending Directives 79/117/EEC and 96/59/EC (Regulation 491, 2002).

5.2.7. *Research and development*

State R&D programs and R&D according to state order are providing the most important platform for research and development concerning POPs issues. State programs are development programs, which should contribute to socio-economic development of Slovakia. They should become the most preferred research tasks in the future, onto which also the financial support should focus. However, general structural problems, caused by a long-term lack of state as well as private financial sources (according to the EU their ratio should be 1/3:2/3) supporting R&D in Slovakia, constitute a potential risk also for implementation of POPs specific project tasks. Besides insufficient financing, also pertaining problems with current legislation, which is not consistent enough with the program financing method pursuant to Law on Science and Engineering, as well as with the Law on Support Agency for Science and Engineering (APVT), are enhancing the risks. Possible tools for implementation of POPs related projects are: utilization of the existing system of R&D financing through APVT (inclusion of POPs into the priority list of the state program of research and development); participation of Slovakia in the 6th EU Framework Program for Science and Engineering or other international cooperation.

The following priorities at the national level were pointed out:

Finalizing the process of adoption of the necessary legal documents for POPs management, setting up the necessary institutional framework and their consistent enforcement; Inventory and environmentally sound destruction of wastes and equipments containing PCBs; Inventory and environmentally sound destruction of obsolete POPs pesticides; Decontamination of the waste water canal from Chemko Strážske; Raising of broad public awareness about hazards connected with open uncontrolled burning; POPs monitoring for the purposes of international reporting, POPs management at national level, and information dissemination to public; Gradual decontamination of all other identified contaminated sites; Research and development in the relevant fields.

5.3. NON-COMBUSTION DEMONSTRATION PROJECT IN SLOVAKIA: DECONTAMINATION OF PCBs

As has been mentioned above, the most urgent and relevant issue to be solved in Slovakia is the environmental contamination with PCBs. For this reason, the Slovak Republic has decided to participate in another global program Demonstration of Viability and Removal of Barriers that Impede Adoption and Effective Implementation of Available, Non-combustion Technologies for Destroying Persistent Organic Pollutants (Short title: Non-combustion Project).

Non-combustion Project is a part of a global program and is primarily oriented towards assistance with PCB stockpile and other POPs wastes destruction using non-combustion technology of high technical level. Technology unit delivery and installation will be supported by the Project grant. Establishment of the conditions for environmentally safe decontamination of polluted sediments and soil is an integral component of the project efficacy. In this demonstration project, UNDP acts as an implementation agency and UNIDO as an executive agency. During the demonstration phase of the project, destruction of an initial volume (1,500 tons of PCB wastes) will be carried out. Project duration together with a preparatory phase, including 2 years of technology performance and results dissemination activities is suggested to be 4 years. The project will be performed in collaboration between public and private sector. Slovak Republic co-financing is aimed on the effective project support, site preparation, operation of the unit, destruction of PCB stockpile etc. (Murín, 2004).

At present, the project document and detailed materials were revised, with the aim to satisfactorily address all GEF Council members' comments. Within the scope of the project document, project technical and financial efficacy, securing of co-financing and roles of the stakeholders should be defined.

Realization of the *Non-combustion Project* will positively affect the PCB decontamination program and PCB-containing equipment phase-out in the Slovak Republic. Besides other measures to be undertaken, safe destruction of PCBs by the year 2010 is required of the EU Member States.

Under the terms of the project, a unit for PCB extraction from the solid matrix (sediment and soil) and a unit for the destruction of PCBs will be provided by the GEF grant:

PCB destruction in a destruction unit will have a projected capacity of 750 tons a year. Sediment treatment includes environmentally safe sediment excavation, storage close to the extraction unit, drying of sediment, extraction and transportation of the extract to the place of final destruction. Cleaned sediments will be placed at the waste dump taking into consideration the residual PCB concentration up to 1 – 2 ppm (mg/kg).

5.3.1. *Production and properties of polychlorinated biphenyls (PCBS)*

In Slovakia, PCBs were produced (21,482 t, in total) at the factory Chemko Strážske in 1959–1984 as commercial PCBs mixtures under the brand names Delor, Hydelor, and Delotherm (Figure 5.1). About 46 % of the PCB production was exported mainly to former East Germany. The rest (11,613 t) was used in the territory of former Czechoslovakia (ca. 5,500 t in SR, 6,000 t in CR) as dielectric fluids for transformers and power capacitors, as heat exchanger and hydraulic fluids, as paint additives and lubricants.

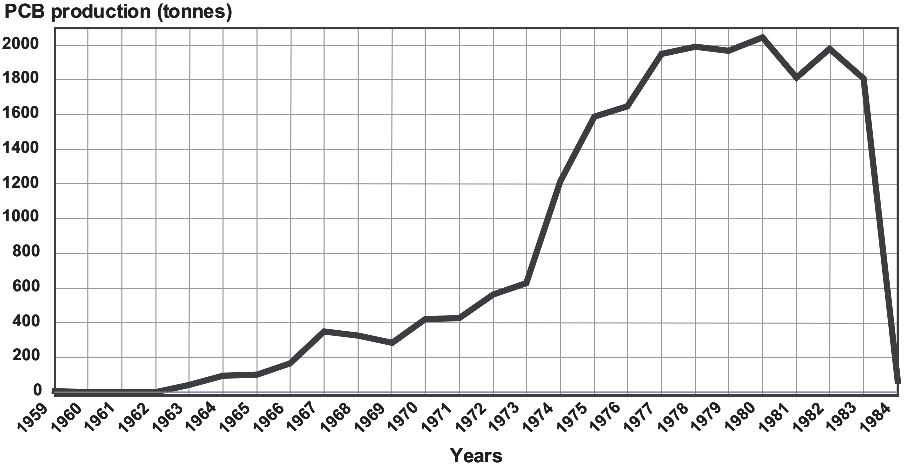

Figure 5.1. Amounts of PCB formulations manufactured by Chemko Strážske in 1959-1984.

PCBs are oily liquids, which contain two aromatic rings, and according to a location of the chlorine atoms they can occur in up to 209 different chemical compounds combinations (congeners), which have different toxicity. PCB production started in the USA 1929 and PCBs have found a broad range of applications in industry (transformer contents, capacitor contents, hydraulic liquids, heat transfer medium, plasticizers, lubricants, impregnates, dyes, glues, additives to construction materials, sealing liquids, burning inhibitors, pesticides etc.). PCBs are emitted into atmosphere via evaporation from free materials and refuse heaps contaminated with PCBs. In water, they are generally contained in sediment which has strong adsorption properties. In soils, they have no tendency to a noticeable spread due to adsorption and low solubility. PCB degradation is very slow – the more chlorinated substance, the slower is the degradation. Biodegradation by microorganisms is slow as well. PCBs can also get into the plants, and some plants have ability to accumulate PCBs. From plant food and also directly from the sea-water, these substances can get into animals.

In humans, PCBs are cumulated mostly in adipose tissue and can be gradually long-term released from adipose cells into blood. The important fact is also, that they are contained in mother's milk, so these toxic chemicals can be received already by newborns. Besides, the health effects are also ascribed to a simultaneous content of dioxin-like compounds, such as PCDFs, in mixtures. Toxic effects are assumed responsible for respiratory, digestive, and liver functional disorders, neurological and process changes (growth inhibition of children, low birth weight, psychomotor process arrest, as well as a possible intelligent quotient decrease). They may also lead to changes in skin pigmentation and to rashes of acne type. There was no carcinogenicity for humans found, however, PCBs are considered to be potential carcinogens. Finally, PCBs are referred to as the substances that impair human immune system, so-called endocrine disruptors.

5.3.2. *Situation with the Slovakia's inventory of POPS: PCB stockpiles*

According to the available data (PCB-containing equipment inventory in years 2001 – 2003), total amount of PCB wastes and material in Slovakia is estimated to be 3,500 tons. Out of those:

- 1,000 tons PCB wastes from Chemko Strážske company (residues and wastes from production): various wastes from PCB production, contaminated clothes, waste material etc. is kept relatively safely in premises inside the factory. The major part is comprised of hardly manipulable material of a semifluid consistence.
- 1,000 tons PCB-containing equipment – transformers, capacitors and other equipment: at present, there are registered: 400 pieces of transformers, 30,000 pieces of capacitors, and 400 pieces of other equipment.
- 1,500 tons various wastes: these originate largely from the agricultural sector and include stockpiles of contaminated hydraulic and transformer oils with PCB contents, scraps of PCB - dyes, contaminated concrete pieces, PCB-containing equipment etc.

Additionally to the documented quantity, it is estimated that at Pláne waste dump there are additional ca. 900 tons of PCB contaminated wastes from production.

In the near future, new legislation will be applied in Slovakia focused on PCB wastes and equipment management. Thus, that total waste volumes will be finally higher comparing with the current estimates. The results of the pilot inventory of PCB formulations used in Slovakia are presented in Figure 5.2 (Kočan et al., 1998).

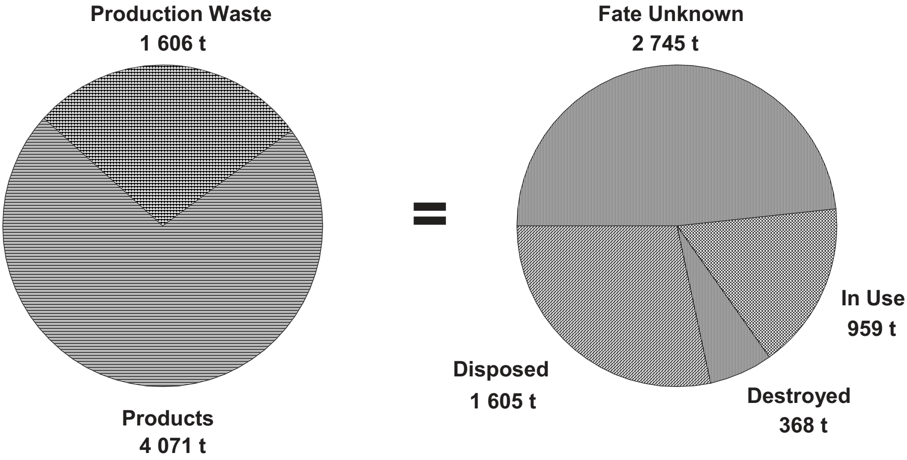

Figure 5.2. Inventory estimation of PCBs used in Slovakia.

5.3.3. *Possible sources of POPs*

In Slovakia, PCBs are still used in power capacitors. Such capacitors can contain from 1.4 to 20 kg of PCBs. As their casing is fragile, PCBs from damaged devices can easily enter the environment.

It seems that the fillings of transformers and heat exchangers operating in Slovakia have already been refilled with non-PCB containing fluids. However, in the past, especially in the case of the heat exchangers, a large amount of PCBs leaked during their operation. Several tens (hundreds) of tons of PCBs were disposed of by incineration either in cement kilns in Slovakia or abroad in hazardous waste incinerators.

Because PCBs were also used as a paint additive (their content was up to 21%), old paint coatings can also release these pollutants.

A layer of mud in the 5.3 km long Strážsky Creek which an effluent canal of the former Slovak PCB producer flows into, containing 15 years after the termination of the production about 3 kg PCBs in 1 ton of dry mud, represents serious environmental source of PCBs. This sediment is an abundant PCB source causing the long-term contamination of the waters of eastern Slovakia (Kočan et al., 1999). Figure 5.3 shows PCB concentrations determined in sediment samples taken in the polluted area (Michalovce District) and in a comparative one (Stropkov District). As expected, the highest value was found in a muddy part of the Strážsky Creek (one of the samples contained up to 5 g/kg, i.e. 0.5%). It is doubtless that the polluted creek emptying into the Laborec River has caused the contamination. The Zemplínska Šírava water reservoir (33.5 km^2 surface area) that is partly filled from the Laborec River contained several hundred times higher PCB levels (100-2000 times) in comparison to a similar water reservoir

(Domaša) in the comparative area. Basing on these findings, one can estimate that at least tons of PCBs are still adsorbed in the sediments of the effluent canal, Laborec River and Zemplínska Šírava water reservoir.

Waste disposal sites are potential sources of environmental contamination. PCBs determined in soil samples collected in the neighborhood of the waste disposal sites of Chemko and plants manufacturing asphalted gravel contained PCB levels much higher than those found in agricultural and forest soil taken in areas remote from possible sources. The evidence of this is provided in Figure 5.4. Those factories often situated in the vicinity of the quarries, i.e. in the mountains on rocky ground, have been manufacturing gravel coated with asphalt to be used for road construction. It is known that due to untightness in heat exchanging systems filled with PCBs, hundreds of tons of PCBs leaked out. PCB concentrations peaked at 53,000 mg/kg in a soil sample taken under one of the heat exchangers. However, high PCB levels were also observed in agricultural (35 and 38 mg/kg) and forest soil (3.9 and 7.5 mg/kg) taken near the plants. As the soil in the vicinity of a major Chemko site, which could be contaminated only by air transported particles, contained increased PCB levels (0.4 to 5.8 mg/kg), it is likely that this waste dump may contain large quantities of PCBs. Similarly, PCB waste might be present at the municipal dump of Michalovce (district town about 20 km from Chemko), since increased PCB concentrations were found in a sample collected close to the dump (0.17 mg/kg).

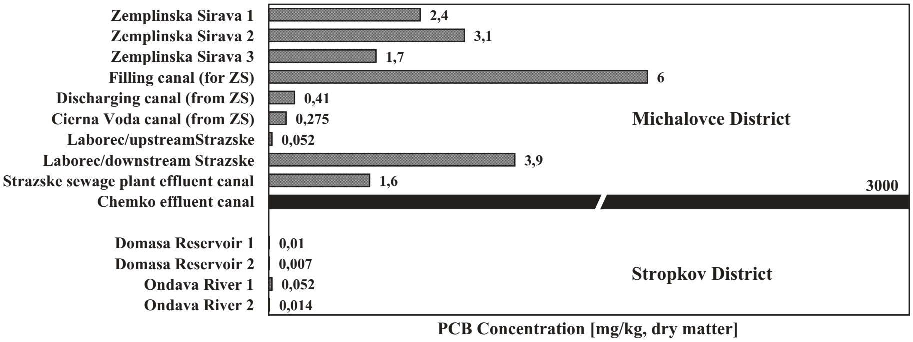

Figure 5.3. PCB levels (the sum of all congeners) in bottom sediment samples taken from some watercourses in the districts of Michalovce and Stropkov.

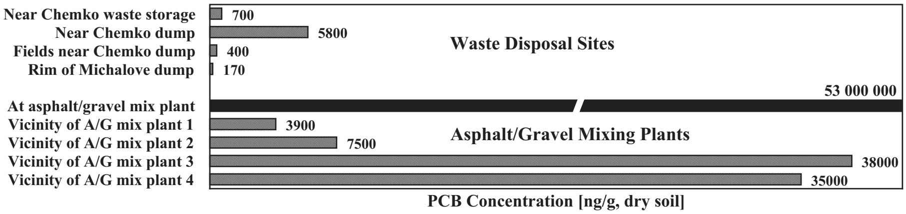

Figure 5.4. PCB levels (the sum of all congeners) in spill samples collected in the vicinity of asphalt/gravel mixing plants and the waste disposal sites of the Chemko chemical factory.

5.3.4. *PCB contamination in Slovakia*

Contamination at the factory Chemko Strážske surroundings belongs to the so-called "old environmental burdens". It directly relates to the former PCB production. Contaminated areas are found inside the factory area and in wider surroundings as well. The contamination is primarily spread through surface water by gradual release from contaminated sediments of an open sewer that leads from the factory to Laborec River, and subsequently through the inlet canal contaminates the water reservoir – Zemplínska Šírava Lake. The contamination is also slowly reaching other areas located lower in the direction of river flow.

Area of contamination by PCBs is partly documented by the previous studies under the project *Environmental and Human Population Stress in Area contaminated by PCBs* (Kočan et al., 1998), which was financed by ME SR. Recently, a study co-financed by WHO has been completed, and data concerning PCB exposure of population is prepared for publication. PCB concentrations in industrial canal fluctuate from grams to tens of grams of PCBs per kg of sediment dry weight, in some places in Laborec at hundreds of milligrams and in sediment of Zemplínska Šírava in milligrams per kg of sediment dry weight. Another problematic area is a waste dump Pláne, where in accordance with the information from Strážske municipality, up to 900 tons PCB wastes could be stored. PCB waste was dumped there during a period of production at Chemko factory. Complex monitoring of PCB contamination in the area surrounding a former production site was not carried out until now. Only partial data exists. For this reason, it is not possible to estimate exactly the amount of contaminated sediment and soils in precise concentration layers. Initial approximation assumes that the open industrial canal may contain up to 40,000 tons highly contaminated material. For final problem resolution of soils and sediment contamination, it is needed to develop a complex strategy considering various concentrations of PCB contamination, technical and economical decontamination aspects, and the timetable.

Water pollution has resulted in a big difference of PCB levels between fish caught in Michalovce waters and fish from a comparative area (Stropkov District). The PCB pollution of Michalovce District was manifested also in the exposure of home-raised animals resulting in increased PCB levels in their meat or eggs. It should be stressed that, in general, PCB levels in Slovak human population (not only of that of polluted area) are higher than those in other countries (with the exception of CR).

PCB contents of the sediments from water bodies from other areas of Slovakia range between 0.01-2.8 mg/kg. Samples with high content of PCBs come mainly from the areas of high industrial activities. The measurements conducted in 1983-2001 documented that 24% of surface water samples (SHMU, 2003) contained more than 10 ng PCBs/L - a recommended maximum concentration limit.

Sediments analyzed within a study performed in 2002 were sampled from different water bodies from all regions of Slovakia. As expected, the highest values of PCBs were determined in sediments from the industrial effluent canal from the factory of Chemko Co. (Table 5.1). The increased

Table 5.1. PCB concentrations in sediments.

	plan-PCBs	**mono-ortho PCBs**	**PCBs**
	[WHO-TEQ, pg/g$_{D.W.}$]		[mg/kg$_{D.W.}$]
Ondava (upstream from Bukocel Co.)	0.17	0.032	0.019
Ondava (downstream from Bukocel Co.)	0.53	0.11	0.027
Ondava (downstream from Kijov. Creek)	0.81	0.16	0.040
Effluent canal (downstream from Chemko Co.)	230	110	109
Effluent canal (downstream from WWT plant)	1960	1240	733
Effluent canal (road Strážske-Michalovce)	1990	1040	567
Laborec (Voľa)	11	6.0	1.4
Laborec (Nacina Ves)	6.8	3.6	0.97
Laborec (Petrovce)	710	430	98
Zemplínska Šírava (Biela hora)	53	20	7.3
Zemplínska Šírava (Medvedia hora)	74	22	5.5
Zemplínska Šírava (Kusín)	27	7.6	2.3
V. Domaša (dam)	0.2	0.039	0.036
V. Domaša (Holčíkovce)	0.16	0.029	0.016
V. Domaša (Nová Kelča)	0.16	0.038	0.011
Nitra (upstream from NChZ Co.)	0.26	0.05	0.022
Nitra (downstream from NChZ Co.)	0.41	0.11	0.13
Nitra (Chalmová)	0.57	0.11	0.074
Váh (Lisková)	1.1	0.64	0.012
Váh (Černová)	1.0	1.3	0.20
Váh (Krpeľany reservoir)	0.46	0.23	0.045

values were also observed in sediments from the Laborec River and Zemplínska Šírava. Other watercourses (Nitra and Váh) showed PCB values at the background levels in accordance with those determined in the previous study (Kočan et al., 1999).

From the comparison of water samples from Zemplínska Šírava and other Slovak water bodies collected in 1997/98 and 2002 one can evidently conclude that only the samples taken in the surroundings of the former PCB producer reach values above the recommended limits for surface waters. Although the PCB production was ceased almost 20 years ago, the industrial effluent canal from the factory (Table 5.1) is the most probable source of contamination with PCBs in this area. PCBs accumulated in sediments are only slowly mobilized into water. This situation will last probably until the start of remediation (Petrík et al., 2001).

5.3.5. *Hot spots and urgent problems*

As regards PCBs, the established highly polluted sites, such as the former producer's effluent canal and some watercourses contaminated by this canal (Laborec River and Zemplínska Šírava water reservoir) can be considered as hot spots and present an urgent problem in Slovakia. Since estimated tens of tons of PCBs are adsorbed in the sediment of these water reservoirs, a flood, for example, might cause an ecological disaster. A possible solution involves the detailed examination of an extent of the pollution of the water reservoir, the removal of the contaminated sediment and the decomposition or retrieval of PCBs by a suitable technology, e.g. incineration, thermal desorption, chemical dehalogenation, solvent extraction, or bioremediation.

5.3.6. *The state of legislation*

In Slovakia, it is prohibited to use PCBs in open systems, however, in the closed ones such as capacitors or transformers, they still can be used. At present, there are legally established limit values for: ambient air, work atmosphere, soils, irrigation water, surface water, foods, and feeds. According to the Directive 473/2000 of Code of Laws of the Slovak Government waste oil containing more than 10 ppm PCBs/PCT shall not be combusted (except cement kilns where exists 50 ppm limit). Waste containing PCBs at range of 50 to 150 mg/kg can be burnt in cement kilns at specified conditions.

In the individual countries there are different limits for specific components of environment and for foodstuffs. In Slovakia, the highest admissible concentrations in working atmosphere are stated in amendment

No. 7/1978 of Ministry of Health of SR official bulletin (average 8-hour = 0.5 mg/m^3, boundary = 1 mg/m^3). For free atmosphere, the maximum average daily concentration of PCBs is 0.08 µg/m^3 (IHE Prague, 1991). Since the primary way of PCB penetration into humans is through food, the content of selected individual PCB congeners was limited in Food Code of Slovak Republic (act of MH SR and MFA SR No. 981/1996 quoted in the official bulletin of MFA SR No. 7/1996) for appointed food commodities (milk and milk products, baby and children food, various meat types, eggs, fish and fish products). In 1990, WHO established a tolerable daily intake (TDI) of 10 pg/kg body weight/day for 2,3,7,8-TCDD, which was revised in 1998 to 1-4 pg/kg$_{bw}$/day for dioxins and dioxin-like PCBs expressed as WHO-TEQ. The upper range pf the TDI of 4 pg TEQ/kg$_{bw}$/day should be considered as a maximal tolerable intake on a provisional basis and the ultimate goal is to reduce human intake levels below 1 pg TEQ/kg$_{bw}$/day.

5.3.7. *Available technologies and facilities for PCBS and pesticides destruction*

In Slovakia, there is no hazardous waste incinerator able to meet criteria for the thorough decomposition of POPs. As already mentioned above, PCB-containing waste (50-150 mg/kg) is allowed to be burnt in cement kilns. The former Slovak producer of organochlorine pesticides can perform decomposition of limited amounts of organochlorine pesticides by alkaline dechlorination. Bioremediation technologies are currently in the stage of laboratory or pilot experiments.

5.4. RESEARCH AND DEVELOPMENT: BIODEGRADATION AND BIOREMEDIATION OF PCBS

The research at Laboratory of Environmental Biotechnology, Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology has been focused on theoretical aspects of biodegradation of chloroaromatics, namely on the factors that should be preferentially considered when looking for strategies how to improve their biodegradation.

The goals of our work can be summarized as follows: 1. isolation and development of PCB degrading microorganisms with a high degradation and survival potential; 2. to design a PCB distribution model describing the concurrent processes of evaporation, biosorption, and biodegradation in a suspension of PCB-degrading bacteria; 3. to use the distribution model for the determination of the primary biodegradation rate constants of individual PCB congeners, in active bacterial suspension; 4. to conduct an empirical structure-degradability analysis of the obtained data; 5. to improve aerobic biodegradation by chemical oxidation; 6. to enhance the efficacy of PCB degradation using naturally occurring inducers; 7. to study biodegradation of PCBs in soil; 8. to study adsorption-desorption properties of chlorinated POPs during their immobilization on organomineral complexes (lignite humic acids bound to zeolite) as an alternative decontamination method; 9. to study effect of aromatics and chloroaromatics on the membrane lipids and fluidity of bacterial cells (Dercová et al., 1995, 1996, 1999, 1999, 1999a, 2001; Tandlich et al., 2001; Vrana et al., 1995, 1996, 1996a, 1998; Dercová et al., 2003, 2004).

Biodegradation of PCB using degradation activity of microorganisms (biostimulation and/or bioaugmentation strategy) represents a more ecological and economical way of PCBs elimination in comparison with a combustion process in contaminated localities with low concentration of contaminants. It is possible to suppose that the improvement of biodegradation in localities with higher contaminant concentration can be reached using a combination of physico-chemical and biological processes.

Acknowledgements

Financial support from the Scientific Grant Agency of Ministry of Education SR (Grant No. 1/1309/04) is gratefully acknowledged.

6. PCBs in sediments of the Zemplinska Sirava Reservoir and the Laborec River in vicinity of Strazske

In this section the results of sediment quality monitoring in the Zemplinska Sirava Reservoir in 2003 are presented, which are compared with those ones obtained in years 1997 – 1999. Furthermore the results of sediment quality in vicinity of Strazske from 2004 are evaluated. The presented outcomes show that bottom sediments in the Zemplinska Sirava Reservoir are substantially contaminated by PCBs, namely by Delors 103 and 106 and by PCB congeners. The values were measured in the order of thousands μg/kg. In comparison to sediments from the reservoir especial attention is paid to PCBs content in sediments from Strazsky Canal. The values from Strazsky Canal were one order of magnitude higher (in the order of ten thousands μg/kg) than the values in sediments of the Zemplinska Sirava Reservoir. The results are fully confirmed by other authors, where PCBs content has been measured on comparable or even higher concentration level.

6.1. INTRODUCTION

The Water Research Institute Bratislava (Slovak Republic) had been dealing with sediment quality of the Zemplinska Sirava Reservoir within the national scientific and technical project (STP) for three years (1997 – 1999) (Hucko et al., 1999a). Obtained results were published, or used for proposal of sediments disposal (Hucko et al. 1999b; Hucko et al., 2000; Hucko, 2001; Hucko and Sumna, 2000). This issue was handled again in 2003 in connection with a verifying of methodical procedures for sediment disposal from water reservoirs (Hucko and Sumna, 2003) and with additional surface water and sediment quality monitoring of selected specific organic pollutants as well (Makovinska, 2004). The results were published in 2004 (Hucko, 2004). In the same year the attention was also focused on bottom sediment quality in vicinity of Strazske (Laborec – Krivostany, Laborec – Petrovce and the Strazsky Canal) (Hucko et al., 2004; Hucko and Kusnir, 2005, Kusnir and Hucko, 2005).

The Zemplinska Sirava Reservoir is situated at the Laborec River at southern piedmont of the Vihorlat Mountain. Its function is to secure a sufficiency of water for the Vojany Thermal Power Station and for irrigations. Another function is also a flood run-off regulation at the Laborec River and partly in area of the whole Eastern Slovakia Lowland. The further aim is a relatively strong application for holiday and recreation (Hucko, 2001). The rest of the reservoir attributes is given in Table 6.1.

Table 6.1. Basic attributes of the Zemplinska Sirava Reservoir.

PARAMETER	UNIT	VALUE
Reservoir Surface Area*	km^2	32.8
Length*	km	11.0
Width*	km	3.5
Depth - maximal	m	14.0
Depth - average	m	9.5
Total Capacity	mil. m^3	334.0
Altitude*	m above see level	117.5
River Basin Surface Area	km^2	1 400.0

* By maximal working level

According to last measurement, the quantity of sediments in the Zemplinska Sirava Reservoir has been approximately 6.9 million m^3 (Hucko and Kusnir, 2005).

The reservoir was substantially polluted by waste waters from the whole river basin in the past and even though significant measures by individual producers of pollution were taken, this load has been persisting till now.

The pollution played the important part on a decrease of quality of surface water and ground water in the river basin. The great progress in improvement of the Laborec River water quality protection was achieved by waste water cleaning technology in the Chemko Strazske enterprise. In those time, own waste water treatment plant was built and diversion of cleaned waste waters off the river basin was applied (into to the Ondava River through the Posa sludge storage lagoon). Only a small part of waste waters (non-contaminated cooling waters, precipitation waters, specific portion of domestic sewage) are discharged via two emergency holding tanks by the outlet canal to the Laborec River. This polluter is emphasized on this spot because of his important participation in the contamination of the Laborec River and the Zemplinska Sirava Reservoir in the past.

6.2. METHODOLOGY

Samples were taken from the Zemplinska Sirava Reservoir in period 1997 – 1999 and also in 2003, during which time they were taken from 4 cross profiles. Sediments from the Strazsky Canal were sampled in 1999 and in 2004 (close to the Vola village).

In case of sediments from the Laborec River, sampling was performed in July and October 2004 at 2 sampling sites:

Krivostany (above the outlet of the Strazsky Canal to the Laborec River)

Petrovce (below the outlet of the Strazsky Canal to the Laborec River)

Overview of all sampling sites is presented in Figure 6.1.

The sediment corer sampler from UWITEC Corp./AUSTRIA was used as a sampling device (see Figure 6.2), which was a transparent plastic tube allowing a visual examination and also a sample partition by layers.

After sample processing these parameters among many others were determined:

Delor-103 and Delor-106 in the area 1997 – 1999,

PCB Congeners # 8, 28, 52, 101,118, 138, 153, 180, 203 in 1999, 2003 a 2004.

Selection of the monitored parameters was done according to the Guideline of MoE SR No. 549/98-2 Risk Assessment of Contaminated Sediments of Rivers and Water Reservoirs and Act No. 188/2003, on Application of Sludge and Bottom Sediments on Soil (Guideline of MoE, 1998; Act No. 188/2003).

The aim of the guideline is to unify the procedures in risk assessment arising from influence of sediment on qualitative composition of upper bed water column what represents water in surface watercourses and water reservoirs.

The act 188/2003[13] specifies conditions of application of wastewater sludge and bottom sediments on agriculture soil and forest soil so as to exclude their detrimental effect on soil nature, plants, water and on human and animal health.

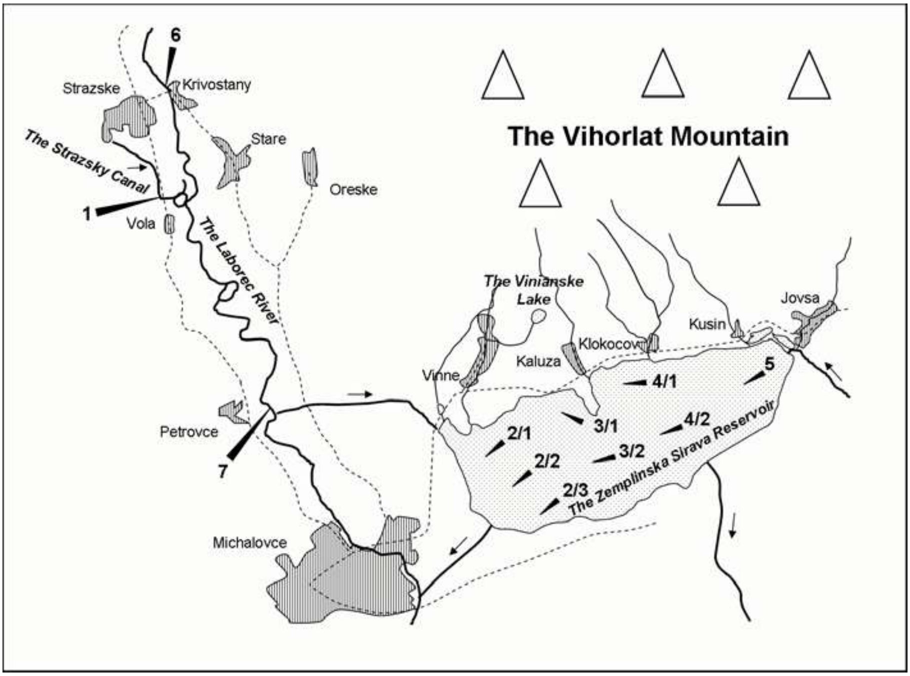

Figure 6.1. Location of the sampling sites in the Zemplinska Sirava Reservoir (ZSR) and vicinity of Strazske

1 – The Strazsky Canal
2/1, 2/2, 2/3 – West part of the ZSR (cross profiles in 2003)
3/1, 3/2 – The ZSR - Stone-pit locality (cross profiles in 2003)
4/1, 4/2 – Middle part of the ZSR, Kaluza (cross profiles in 2003)
5 – East part of the ZSR, Kusin
6 – Laborec – Krivostany
7 – Laborec – Petrovce

Figure 6.2. Sampling device Corer.

6.3. RESULTS AND DISCUSSION

6.3.1. *Sediment quality in the Zemplinska Sirava Reservoir*

In the past there was monitored Delors occurrence (D-103, D-106) in a larger measure in this reservoir (in water and sediments), because of their importance with regard to the Slovakian industry production and use. That is the reason why they were monitored first of all on the beginning of our trials. In 1999 and continuing in 2003, the monitoring was extended by other PCB congeners, which had started to have a higher environmental importance (PCB 8, 28, 52, 101, 118, 153, 180 and 203) (Guideline of MoE, 1998).

When we compare all sampling periods in the Zemplinska Sirava Reservoir in years 1997-1999 and 2003, the highest values of D-103 and D-106 were detected in 1999, whereas this kind of analysis was not already performed in 2003.

With regard to a sum of PCB congeners it was approximately three-fold higher in 2003 in comparison with these values from 1999. The highest value was measured for congener # 180 in 1999 and for congener # 118 in 2003.

It is necessary to be stated that here presented (compared) values are averaged from all measurements obtained in respective sampling period on the Zemplinska Sirava Reservoir (e.g. all values obtained from the individual cross sections from 2003 were put into average one).

6.3.2. *Sediment quality in vicinity of Strazske (1999)*

The results of PCBs sediment analysis from the Strazsky Canal (close to Vola village) showed, that here measured values were a few orders of magnitude higher than values from the Zemplinska Sirava Reservoir (sum of congeners in the canal 65 619 μg/kg towards 1 124.18 μg/kg in the reservoir). Delors (D-103 and D-106) in sediments from the Strazsky Canal highly exceeded the values from the reservoir (D-103 76 852 μg/kg and D-106 57 097 μg/kg towards 734.2 μg/kg for D-103 a 985.9 μg/kg pre D-106). The results are fully confirmed by other authors, where PCBs content has been measured on comparable or even higher concentration level (Petrik et al., 1999).

6.3.3. *Sediment quality in vicinity of Strazske (2004)*

In addition to the Strazsky Canal, the results of sediment samples analysis from the Laborec River in Krivostany and Petrovce were also evaluated.

PCB congeners were detected in sediments of the Strazsky Canal in July (34 765 μg/kg) and in October (18 644.6 μg/kg) as well. This detection was also confirmed in sediments from Krivostany in July at 1 451 μg/kg (congeners # 52 and # 153 – 1 180 μg/kg) and similarly in October at 429.9 μg/kg (congeners # 52 and # 153). Values being measured at these localities were 24 and 43-fold lower in comparison with those ones in the Strazsky Canal.

There were also detected higher PCB congener's values in the Petrovce sampling site (1701.4 μg/kg) in comparison with Krivostany and lower ones in relation to the Strazsky Canal. The results show the highest values at congeners # 8 and # 28.

In case of suspended matters (silt content), which were sampled in July, lower PCB congeners values were measured (102.3 μg/kg), in relation to the remainder.

Obtained results of PCBs content in analysed sediments and suspended matter are showed in Figures 6.3 – 6.5.

In order to focus our research not only on monitoring of sediment quality, in the process of leachibility (desorption) and mobility we tried in laboratory condition to characterize the retroactive impact and behaviour of selected organic compounds accumulated in sediments in the water/sediment interface. Among others we used a source of contaminated sediment by PCB from the Strazsky Canal (July 2004) and also from the Laborec Petrovce (October 2004).

As we found out, PCBs together with selected polycyclic aromatic hydrocarbons (PAHs), non-polar extractable substances (NES) and hexachlorobenzene (HCB) showed very poor leachibility confirming their strong binding on sediment particles[9, 10, 11].

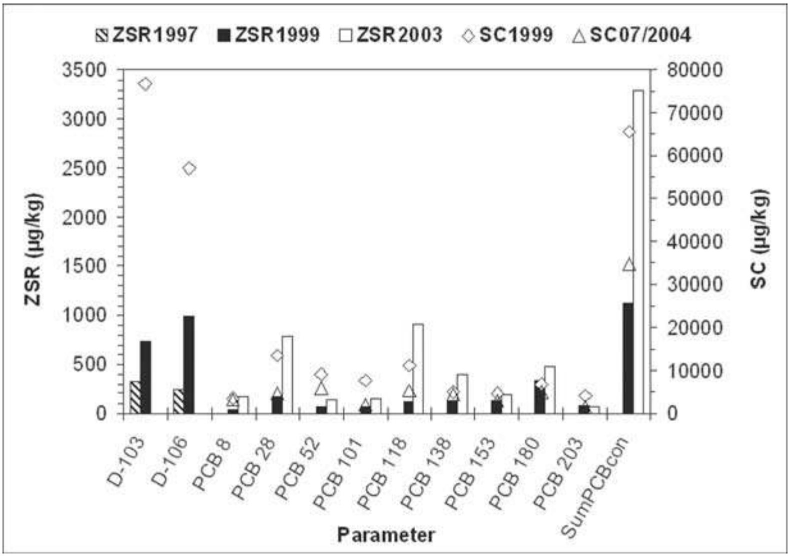

Figure 6.3. PCBs in sediments of the Zemplinska Sirava Reservoir (ZSR) and the Strazsky Canal (SC).

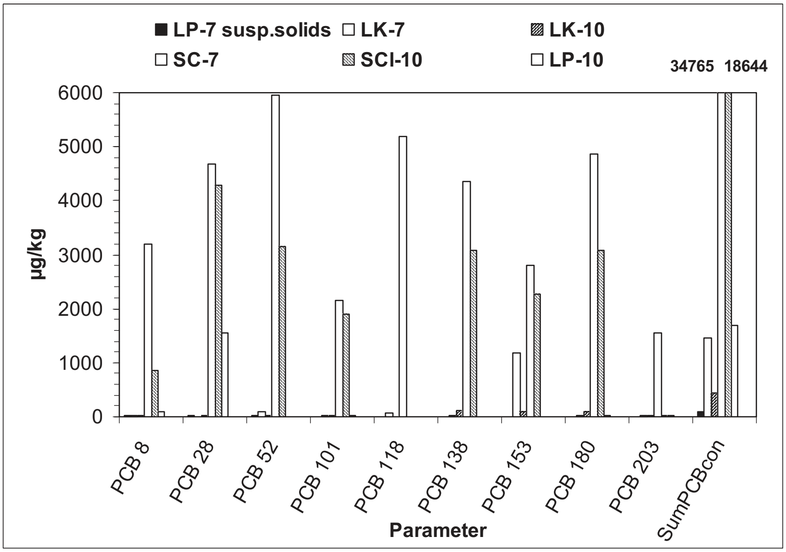

Figure 6.4. PCBs in sediments in vicinity of Strazske in July (7) and October (10) 2004: Laborec - Petrovce (LP), Laborec - Krivostany (LK), the Strazsky Canal (SC).

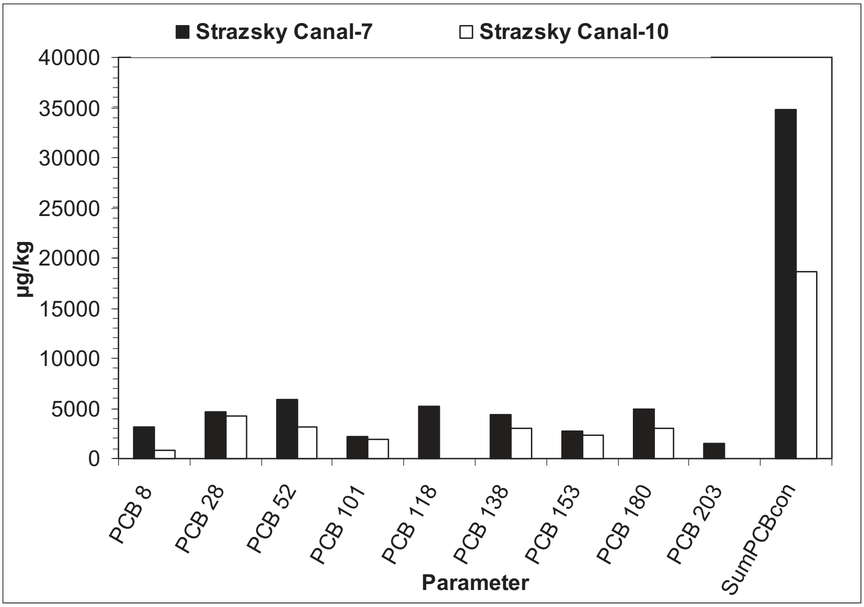

Figure 6.5. PCBs in sediments of Strazsky Canal in July (7) and October (10) 2004.

6.3.4. *Conclusions*

The PCBs in sediments from the Strazsky Canal reached the values being a few orders of magnitude higher than those values from the Zemplinska Sirava Reservoir.

There was shown a significant degree of pollution by PCBs, namely by Delors D-103 and D-106 and by PCB congeners in sediments of the reservoir relative to sediments in the canal (such substantial degree of pollution did not appear for the rest of parameters, e.g. heavy metals, PAHs).

The higher content of PCB congeners was detected by an increased water stage during flood period of the Strazsky Canal, which could have been probably caused due to PCBs washing out of the bed.

In 2004 the set of laboratory experiments with sediments from the Laborec – Petrovce and the Strazsky Canal was performed. In the process of leachability and mobility, the behaviour of PCBs accumulated in these sediments in the water/sediment interface was evaluated. There was typical very poor desorption of PCBs (together with other organic compounds) from sediments into water.

7. Recommendations

7.1. WORKGROUP OBJECTIVES

Discussion of the approaches for managing contaminated sediments as described in the preceding section led to recognition for the need to answer the questions:

- What are the *major barriers* to successful sediment risk assessment and management? and
- What are the science *and infrastructure* needs to address these barriers?

This section summarizes the results of that discussion and preliminary efforts to answer those questions.

7.2. BACKGROUND: CONTAMINATED SEDIMENT ASSESSMENT AND MANAGEMENT

The magnitude of the challenge posed by contaminated sediment is large. The U.S. Environmental Protection Agency (USEPA) has estimated that in the U.S. more than 1 billion cubic yards of surficial sediment are sufficiently contaminated so as to pose a potential risk to human health and the environment (USEPA 1997). While relatively little quantitative information exists on the extent of the global scope of the problem, there is little reason to think that the situation in the U.S. is markedly different in other industrialized countries. Europe-wide, the volume of dredged material (for capital, maintenance and remediation purposes) can be very roughly estimated at 200 million cubic metres per year. If the US estimate that at least 10% of dredged material is too contaminated for open disposal, this results in 20 million cubic meters of contaminated dredged sediment. However, given that a large proportion of European rivers and ports are in highly populated and industrialized areas, the proportion can most likely be expected to be higher. Little information exists to allow estimates of the extent of sediment contamination throughout Europe, but emerging data suggest a vast and complex problem, particularly in some of the former Eastern Block nations that are currently joining Europe (Bridges et al. 2005).

Given the role sediments play as both a sink and a source of contaminants in aquatic systems, numerous government and private organizations are working to develop the policies and the technical means to address the challenge of how best to manage contaminated sediments, but the policy, approaches, science and frameworks to address these problems differ vastly both regionally and nationally. Over the last 30 years, several countries have proposed or promulgated environmental laws and regulations that address a broad range of stresses imposed by human activity on the environment. For example, sediment quality is addressed in the U.S. by several state and federal programs and regulations, including most prominently the National Environmental Policy Act, the Clean Water Act, the Marine Protection, Research, and Sanctuaries Act and the Comprehensive Environmental Resource, Compensation, and Liability Act (NRC 1997). Whilst many countries and regions in Europe have or are developing sediment quality standards and assessment programs, the state and details of these differ from country to country. Within the European Union, the Water Framework Directive contains far-reaching provisions intended to secure and manage water resources, and largely by implication, sediments at the river basin scale (Brils 2003; Brooke 2004a; Brooke 2004b), but sediments are not explicitly addressed. It is of note that the WFD does not recognize the important role of sediments in many natural systems, even treating suspended solids as a pollutant, whether contaminated or not. Sediment management is directly or indirectly included in different European directives such as the Waste Directive and Habitats Directive (Köthe 2003). Guidelines for management of coastal dredged material have been developed by several international maritime conventions, including the London Convention, OSPAR and the Helsinki Convention (Bergmann and Maass 2005, in press; den Besten et al. 2003).

Sustainable sediment management will require extensive collaboration and research to adapt current systems of environmental assessment and management to the basin and ecosystem level. Therefore, an integrated, multi-stressor, multi-use/user, multi-media management approach should be developed which encompasses a decision-making hierarchy. This should aim at setting priorities at a basin scale followed by site-specific risk assessment at a local level and control of point and diffuse contaminant sources (Apitz et al. 2005, in press), and other pressures (Landis 2005). A description and model, whether conceptual or quantitative, of the mass flow of water, contaminants and particles within a river basin, or Conceptual Basin Model, (CBM: (Apitz and White 2003), if thoughtfully developed, is a critical part of effective risk assessment for a particular site, and for basin-scale management as a whole. Such a CBM should also be put in terms of the ecological receptors in aquatic ecosystems, and in terms of other drivers of ecosystem impact (e.g., habitat loss, saline incursion, thermal change, sea level rise, abstraction, and so on). If, by any process occurring within the river basin, contaminants, activities or other pressures pose a risk to ecosystems or river functions at other sites, a control of that risk source is a vital part of an effective management plan of the overall basin.

7.3. BARRIERS TO SUCCESSFUL SEDIMENT RISK ASSESSMENT AND MANAGEMENT

There seemed to be two general approaches to these questions, one can be categorized as the top-down approach (barriers perceived by information users such as regulators and decision makers) and the other as the bottom-up approach (barriers perceived by information generators such as scientists and site assessors). In spite of this, there were very common themes.

- There is a difficulty in establishing links…
    - …between contaminant presence and bioavailability (i.e., between hazard and risk)
        - There is a lack of common strategies/procedures to reliably estimate the actual bioavailability of priority pollutants in sediments and in soils.
            - Some felt that the fact that many sites are prioritized not based upon actual cause-and-effect relationships within an ecosystem but by conservative methods employing conventional sum-parameters for pollutant content determination posed a significant barrier to progress.
            - Others felt that the use of risk-based evaluations (which are often not straightforward) rather than strict sediment quality guidelines (SQGs) impeded progress
            - Some panellists felt that SQGs should be the same internationally (or throughout regions or continents), others felt that there needed to be national (or regional) development and validation.
        - There are no common strategies for addressing non-priority pollutants or the effects of non-contaminant causes of stress such as organic loading, thermal stress, habitat loss, disturbance, etc.
    - …between upstream sources and downstream sinks
    - …between a determination of risk and a decision to manage
        - There are technical, financial and policy gaps between assessment/monitoring and remediation
            - There are no standard methods for prioritizing sites for management. This

        problem is exacerbated by a lack of funds, and a perception that politics, rather than risk, drive prioritization in many cases
      - It is not always clear, once a risk is determined, how to manage that risk (particularly at large scales)
      - In many cases, there is no policy or public commitment to the cleanup of contaminated sites, even if risks are established
  - …between universities (scientists) and agencies (decision makers)
    - Both scientists and decision makers voiced frustration on this front. Researchers felt that decision makers were not using their science, either at all or appropriately, and decision makers felt that scientists were either not carrying out research of relevance to their real-world problems, or they were not communicating the results of that research in terms that made them useful
  - …between various agencies
    - There is a need for various government agencies to develop better communication in support of common policies and goals. This lack of interaction was observed within a given country (either from various regions or with different responsibilities (e.g., coastal, water, soil and river management), and between countries (both within continents or internationally)
  - …between various disciplines (e.g., hydrology, toxicology, chemistry, etc.)
    - Soil, water and sediment management (as well as freshwater and marine sediment management) are treated as unrelated issues, making effective, basin-scale management impossible
    - A particular barrier is the balance and communication between the technical and socioeconomic issues driving sediment management

Scientists and decision makers all pointed to money as the biggest barrier to effective management - there is not enough to fund all relevant research, nor to clean up all sediments. At times, there is not even enough to carry out sufficient monitoring.

7.4. SCIENCE AND INFRASTRUCTURE NEEDS TO ADDRESS THESE BARRIERS

- There is a need for methods, models and policies in support of basin-scale risk management
    - There is a need for more extensive cooperation between the EU Environmental Protection Agencies and a more extensive cooperation between these and the USEPA structures, in order to develop uniform common and unique limits of acceptance of pollutants in sediments
        - (there was no consensus on this statement - others felt the need for national standards independent of those of other nations)
    - There is a need for a comparative evaluation, selection and standardization of a limited group of procedures for estimating the bioavailability of pollutants in sediments
    - There is a need for the development of innovative and effective (bio)analytical strategies for the estimation and monitoring of emerging pollutants, such as hormones, endocrine disrupters, pharmaceutical products, in sediments. This might enhance the potential of actual and future risk analyses procedures
    - There is a need for strategies to identify contaminant sources, predict sediment transport, deposition and erosion within the stream bed considering both continuous and sporadic processes (such as storm events, floods and snowmelt), and how this affects risk distribution at the basin scale
        - There is a need for methods to determine contaminant transport pathways and processes from the sediment to the endangered subject (processes of the contaminant release from the sediment and its transport to risk recipient)
        - There is a need for methods to define sediment vulnerability areas for particular streams (areas from where the contaminant is transported), including possible transport processes – as a way of “source control”
        - There is a need to identify the sediment accumulation sections within the streambeds as well as the final sediment transport barriers (e.g. dams), in connection with the possible contaminant sources

        - There is a need for quantitative Conceptual Basin Models to achieve this, and thus for research on physical system function in rivers, estuaries and coasts
    - There is a need for research into how non-priority pollutants and the effects of non-contaminant causes of stress such as organic loading, thermal stress, habitat loss, disturbance, etc., affect ecosystem status and function, to ensure that management strategies are properly addressing the causes of impact
    - There is a need for interdisciplinary collaboration and communication as well as regional, national and international networks to develop uniform approaches, models, policies and monitoring tools in support of these goals
- There is a need for research in support of standard methods and frameworks, and thus for the development of decision and communication tools that link this complex science to the needs of society
    - There is a need to determine what aspects of sediment risk assessment and management (tools, standards and approaches) are universal, and which require regional or national validation.
    - There is a need for large-scale validations of remedial technologies. These must not just demonstrate site-specific technical feasibility but should provide information that might be generalizable to other sites or nations. For example, in Italy, policy states that they can "inject only substances specifically necessary for remediation scope", but few demonstrations have helped develop tools for such questions.
    - There is a need for common strategies/procedures to reliably estimate the bioavailability of priority pollutants in sediments and in soils, allowing for the prioritization of sites
    - There is a need for transparent, science-based decision frameworks for assessing risk, prioritizing sites, determining the necessity of remediation and selection of the most suitable management methodologies.
        - This should ensure the allocation of funds to sites that require urgent management activities.

- There is a need for these decision tools frameworks to allow for a transparent balance of scientific, technical, regulatory and socioeconomic issues
  - In support of this, there is a need to "translate" science into the language of decision makers

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PHYSICOCHEMICAL AND BIOLOGICAL ASSESSMENT AND CHARACTERIZATION OF CONTAMINATED SEDIMENTS

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Abstract- Physicochemical and biological processes that influence contaminant fate and transport in sediments are diverse, and include physical chemical behaviour, biological processes at microbial and macro scales, as well as pore water and solid phase transport. Spatial and temporal variability in these environmental characteristics and processes are large, and can be difficult to portray and predict. We herein present a series of case studies that evaluate environmental processes and properties that must be assessed with respect to studies of contaminated sediments. These topics are: chemical behaviour of metals in the environment; biological processes and sediment properties influencing contaminant release from sediments; the biogeochemistry and adsorptive capacity of ambient organic compounds in aquatic environments; and new approaches to modeling and scaling the spatial distribution of relevant processes and sediment properties. In a concluding assessment of our state of understanding for these processes and properties, we find that significant knowledge gaps remain in terms of both causal and statistical uncertainty, and these uncertainties should be the focus of new research and development in assessment and characterization of contaminated sediments.

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1. Chemical behavior of metals in the environment

The three main processes that govern the chemical characteristics of contaminant behavior in the environment are: oxidation-reduction reactions, adsorption processes and dissolution-precipitation reactions. These processes also play an important role in the fate of metals. Redox reactions control chemical speciation of many metals affecting their transport in the environment. Although many redox transformations are biologically-mediated as irreversible reactions, the focus in this chapter is on redox and speciation equilibria, particularly how to handle chemical complexity in real systems. Microbiologically-mediated reactions are addressed more comprehensively in Chapter X (Agathos). In the following sections we first present the problems associated with predicting the rates of chemical weathering at mine sites and how to overcome these problems by predicting rates for mineral weathering from laboratory and field data. The second section presents metal speciation and the role of sorption on complex natural sorbents as a control on the mobility of metals within aqueous systems. The final section outlines the value of understanding these processes in the natural environment and the implications for risk assessment.

1.1. MINERAL WEATHERING AS A SOURCE OF METAL POLLUTION

Mining activities worldwide contribute significantly to the solute loads of receiving streams and aquifers. Mining activities contribute with an estimate of 12% to the fluvial sulfate to the sea (Nordstrom and Southam, 1997) and release dissolved metal ions and acidity as potential environmental hazards. Therefore, understanding how fast sulphide minerals generate acidity and metal loads to receiving waters and sediments is crucial. The dissolution of iron sulphide minerals disrupts the acid-base balance in the vicinity of mine sites, as expressed by the iron sulphide (pyrite) dissolution reaction:

$$FeS_{(s)} + 7.5\ O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad (1)$$

Pyrite weathering produces sulfate, releases soluble ferrous iron (Fe^{2+}) and produces acidity, represented in equation (1) by the production of protons. Transformation of soluble ferrous iron by oxidation into soluble ferric iron (Fe^{3+}) needs also be accounted for (Younger *et al.*, 2002). The net impact on the acid-base balance depends on the rates of alkalinity production from dissolution of carbonate and silicate minerals.

At the same time, the dissolution of other metal sulphides such as ZnS(s) (sphalerite) and PbS(s) (galena) releases potentially hazardous metals to the environment, but does not necessarily produce acidity.

$$ZnS_{(s)} + 2O_{2(aq)} \rightarrow Zn^{2+} + SO_4^{2-} \quad (2)$$

$$PbS_{(s)} + 2O_{2(aq)} \rightarrow Pb^{2+} + SO_4^{2-} \quad (3)$$

These chemical processes can be quantified from laboratory data and the associated weathering rates can be calculated by normalizing the data to the mass of reacting mineral or rock sample. A crucial factor to quantify in order to extrapolate weathering rates from laboratory experiments to field scale is the reacting surface area of the minerals at the respective scales of observation (Banwart *et al.*, 2002). The value of specific surface area of a mine site is generally not known, leading to very uncertain estimates of contaminant loadings and contaminant weathering rates at field scale. Surface area values obtained by empirical site-specific estimates at field sites are generally two-three orders of magnitude lower than the specific area determined by laboratory studies (Banwart *et al.*, 2002). The challenge for risk assessment is therefore to develop a generic method to predict chemical processes rates which: (i) determine contaminant source strength and longevity of acidity and metal ions; (ii) attenuate acidity loads through neutralization along transport pathways and (iii) attenuate metal ion loads through sorption and precipitation reactions. Such method will allow the estimation of environmental impact from desktop where limited funding for site investigation or limited data is available. Although research to date has focused on metals release rates from mine sites, the concepts are equally applicable to metals sources, such as dredged sediments or other metals-bearing waste, stored above the water table or deposited in locations with hydrological transport pathways to surface waters.

1.1.1. *Determination of weathering rates by aqueous chemical methods – Prediction of field weathering rates from laboratory data*

A potentially general scaling method to predict field-weathering rates from laboratory data was developed for a mining site at Aitik, Sweden (Malström et al., 2000). The scaling algorithm quantifies for different observations scales (lab vs field) the effects on weathering rates from differences in temperature, physical surface area, mass of flowing water and rock reacting and relative abundance of reactive minerals. For laboratory assessment of weathering, the reactions are generally studied using either of two main experimental approaches, namely, closed batch reactors or flow-through column reactors (see Banwart et al., 2002). Batch reactor experiments are a simple approach to determine the potential of a soil or rock sample to generate contamination. The reactors are set up with a known mass of solid material and a known volume of aqueous solution. The reactor is run under controlled laboratory conditions and parameters such as ionic strength solution, pH and $O_2(aq)$ concentration are fixed. Batch reactors can be set up with large numbers of replicate experiments that can be run over periods of months. The progress of the reaction is followed by periodic sampling and monitoring of chemical composition. In this type of experiment the increasing mass of solutes present in the aqueous phase can be related to the source minerals present. The weathering reaction rate is then determined as moles of mineral dissolving over time. Laboratory rates determined in this fashion can be then used, with an appropriate scaling method such as described above, to predict source strength at field sites.

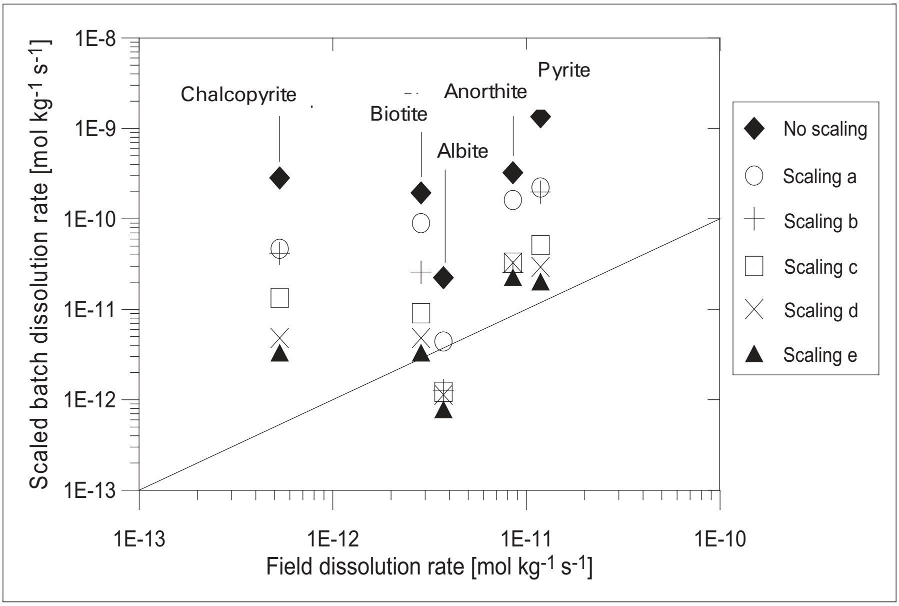


Figure 1.1. Comparison between batch mineral rates and field dissolution rates. (a) Temperature; (b) pore water pH; (c) particle size distribution; (d) mineral content; (e) water flow patterns.

Column experiments may be set up under hydraulically -saturated or -unsaturated conditions, offering the opportunity to simulate realistic solid to water ratios, gas transport such as $O_2(g)$ ingress, and element release as a function of water flow rate. This includes assessing whether solubility equilibria between effluent solutes and source or secondary minerals occurs. It also evaluates whether ion release is controlled by chemical kinetics or by mass-transfer diffusion.

Comparison between mineral weathering rates determined in batch laboratory experiments and field rates are shown in Figure 1.1. The solid lane represents the ideal case where batch test rates equal field rates. Batch rates calculated without taking into account the effect of factors such as temperature, pH, particle size distribution, mineral content or water flow patterns are 2-3 order of magnitude higher than rates determined from field data. Progressively accounting for differences in the factors (a)-(e) at lab and field scale, to a large degree, resolves the discrepancy. These results shows that scale-dependence of these rates is predictable to a large degree by quantification of the effects of a few bulk physico-chemical characteristics of a field site. The fact that this scale dependence is consistent with other laboratory studies (discussed in Malmström et al., 2000) suggests the general applicability of extrapolating weathering rates from the laboratory to many types of field sites.

1.2. REDOX/BIO-GEOCHEMISTRY OF METALS

The main chemical transformation reactions that metals undergo in the environment are oxidation-reduction (redox) reactions. Redox reactions change the speciation and solubility of many elements, create new compounds and affect the biogeochemistry of soils. Redox reactions are not uniform in soil and sediment matrices and depend on the dynamics of soil oxygen and other dominant redox-reactive components. Figure 1.2 shows a scale for some of the major redox couples in the environment. The oxidized form of each element is shown on the left side of the scale and the reduced form on the right. Any oxidized form can react with the reduced form of another couple located below it on the scale. These reactants correspondingly form their reduced and oxidized forms as reaction products.

Of particular importance are reactions that are related to dissolution or precipitation of mineral phases since this transformation radically alters the mobility and bioavailability of these substances. For example, manganese is sparingly soluble under oxic conditions as $MnO_2(s)$ but is reductively dissolved under reducing conditions thus liberating Mn^{2+} to solution. The ion is difficult to remove from mine waters to the concentration levels that are often required by regulatory control (see Younger *et al.*, 2002). Another example is given by metal sulphide phases such as FeS(s) that are sparingly soluble under reducing conditions, but release soluble metals and sulphate ion when oxidized.

A particularly important case is given by the oxidative precipitation/-reductive dissolution of iron oxyhydroxide minerals. These minerals are abundant in mining environments due to release of Fe by pyrite weathering (Eq. 1).

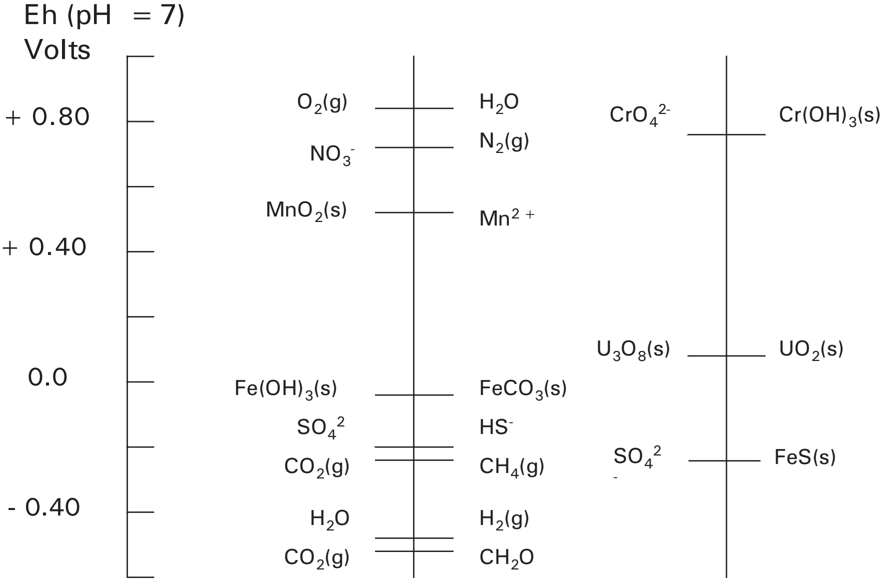


Figure 1.2. Redox ladder showing common redox couples in the environment.

Figure 1.3 shows the Pourbaix diagram for iron and water in soil and sediment with the stability limits for water (see Garrels and Christ, 1965) and the stability fields for various Fe mineral phases and dissolved forms. Since the oxidation of Fe^{2+} to Fe^{3+} is independent of pH a straight line with intercept at 0.771 volts implies that the activity ratio of the two iron species is always 1. There are no redox reactions involved in the transformation of Fe^{3+} into $Fe(OH)_3$ giving a straight line parallel to the Eh axis. At pH of natural environments the iron speciation is dominated by the presence of Fe^{2+} at low Eh values (anoxic environment) and the oxidation and subsequent precipitation of Fe^{2+} into $Fe(OH)_3$ (oxic environment). Other phases as iron -carbonates, -sulfides and silicates can be added to the diagram as necessary for the system considered. With respect to other redox couples, similar diagrams can be constructed for other elements in soil and sediments systems, including potentially toxic metals such as chromium, arsenic or uranium which are affected by redox transformations and changes in pH.

In particular to the case of iron is the role of $Fe(OH)_3(s)$ to act as a strong sorbent for many types of metal ions. The presence of $Fe(OH)_3(s)$ thus acts to scavenge metals from the water column or pore waters, and can release these sorbed contaminants if redox transformation to soluble Fe^{2+} occurs under reducing conditions. In the following session, more attention is given to the behavior of $Fe(OH)_3(s)$ to scavenge metals from solution.

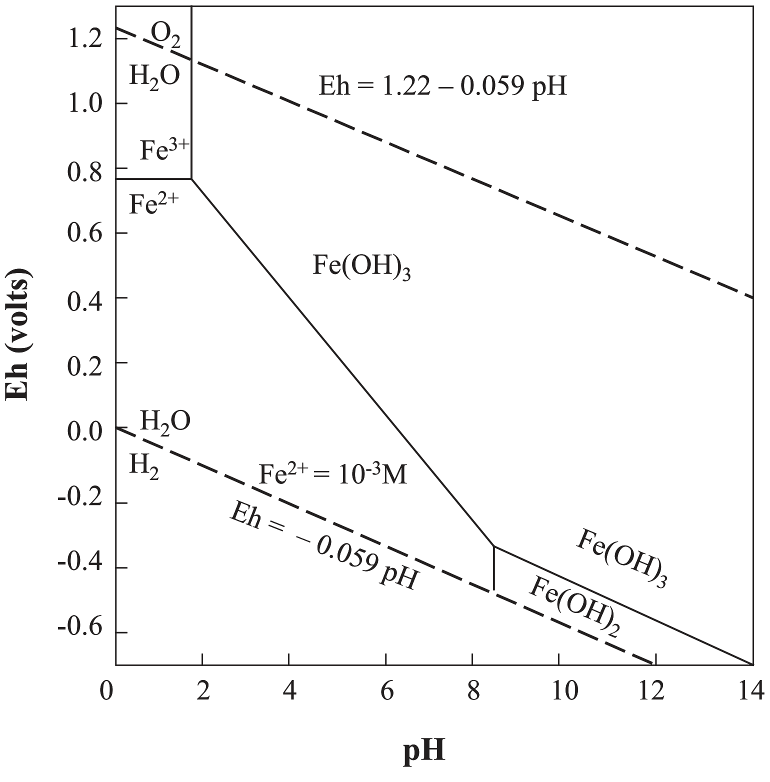


Figure 1.3. Pourbaix diagram for iron in relation to Eh and pH boundaries in soil and sediments.

1.3. SORPTION OF METALS

The ultimate fate of metals released into the environment depends on the transport pathways, the redox dynamics and the chemical transformations occured such as sorption and solubility. As discussed above, in the case of iron, oxidative precipitation produces solid surfaces that may act as sorbent for other metals. In order to assess the contribution of sorption processess on iron oxyhydroxide mineral surfaces to the fate and transport of metals, it is possible to estimate sorption using existing chemical equilibrium models that have been tested in a wide range of laboratory studies (see Dzombak and Morel, 1991).

1.3.1. *Sorption and metal speciation in the presence of iron hydroxide mineral surfaces*

The term species describes the form in which a metal ion is present in aqueous solution. A metal ion may exist in solution as one or more positively or negatively charged species, as an organic complex, ion pair complex, hydrated complex or hydroxide (Stumm and Morgan, 1996). Understanding the distribution and relative concentrations of metal species, for example as a function of pH, is crucial in order to predict the reactions or type of bonding that the metal may undergo in the natural environment. The extent of adsorption on $Fe(OH)_3(s)$ as a function of pH is compared for a range of metal contaminants in Figure 1.4. A key point is that adsorption of all of these metals increase strongly with pH, where the pH threshold for adsorption is unique for each contaminant. Lowering pH can therefore strip adsorbed metals from the mineral surface and lead to release of previously scavenged metals contamination to solution. Another important point is that cationic metals species are able to adsorb strongly on positively charged surfaces. The surface of $Fe(OH)_3(s)$ exhibits variable charge with pH and has zero net charge at circumneutral pH. Below this pH, the mineral surface is positively charged. It is clear from inspection of Figure 4 that Cu^{2+}, Pb^{2+} and Hg^{2+} form dominant surface species at pH values where the mineral surface would otherwise be positively charged.

The results from Figure 1.4 demonstrate influence of pH and speciation in sorption behavior for relatively clean systems that are amenable to study by setting up controlled laboratory experiments with synthetic minerals in clean aqueous systems.

A more general overview of the theory and the methods to study these reactions are presented in Banwart (1997). The experimental design for these types of studies provides information about the kinetics of the reaction, species present and sorption reactions occurring at variable or fixed pH. The advantage of these systems is that they allow quantification

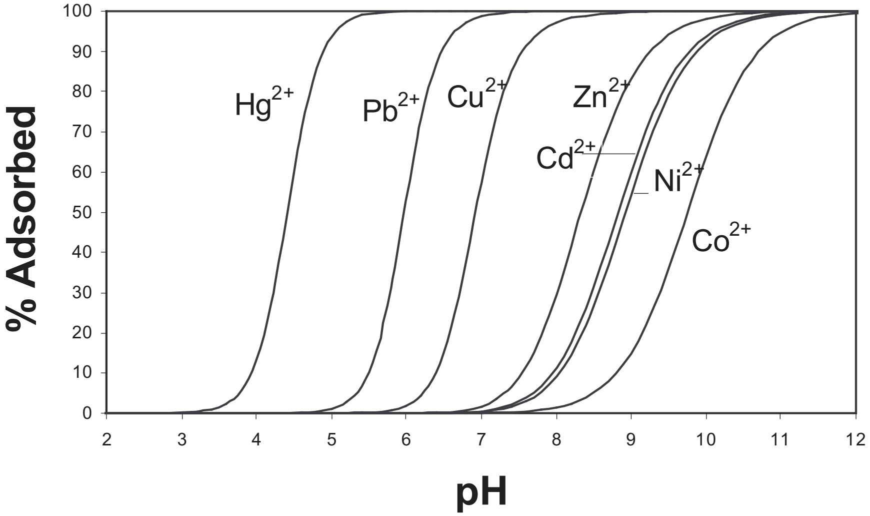


Figure 1.4. Adsorption and acid-base behavior of some common metals in the environment.

of the effect of changing one or more known variables systematically, thus allowing investigators to unravel details of the sorption process occurring in the system. In the following section, a case is presented on how these types of aqueous chemistry studies can be combined with advanced surface analysis techniques based on spectroscopy of micro-imaging, in order to obtain thermodynamic data and detailed speciation and chemical bonding information.

1.3.2. *Sorption and metal speciation in the presence of complex sorbents*

Aqueous chemistry methods described previously are usually sufficient to explain speciation and complex formation in controlled clean systems and as first-order estimates for metals scavenging (or release by desorption) in natural waters if the reaction of interest has been demonstrated to dominate. However, in complex adsorption systems such as in soil, or in biofilms on sediment surfaces, the information provided in the existing literature, most generally obtained from studies on single, synthetic minerals, is often not sufficient to describe the reactions occurring. In this case is therefore necessary to combine aqueous chemistry studies with measurements on the sorbent surface. These are observed as a function of changes in the aqueous chemical composition.

In a recent study, surface spectroscopy has been used along with bulk aqueous speciation methods to elucidate the sorption mechanism of metals to a natural biosorbent (Romero-González *et al.*, 2001 and 2003). Information obtained by potentiometric titrations and isotherm studies provided the average stoichiometry of the ion exchange reaction and the nature of the functional groups. This data allowed investigators to determine the thermodynamic constants for formation of the metal complexes on the surface of the biosorbent. Surface and micro-spectroscopy provided confirmation of the composition and distribution of the functional groups and metal-ligand stoichiometry. It also provided information on the metal coordination environment and bond distances. In turn, a chemical structure of the metal-biosorbent complex may be proposed and metal-binding preferences can be predicted. Figure 1.5 shows the proposed structure for Cd^{2+} sorbed to dealginated seaweed waste, a biosorbent that is being studied for its potential as a sorption barrier and waste treatment material for metals.

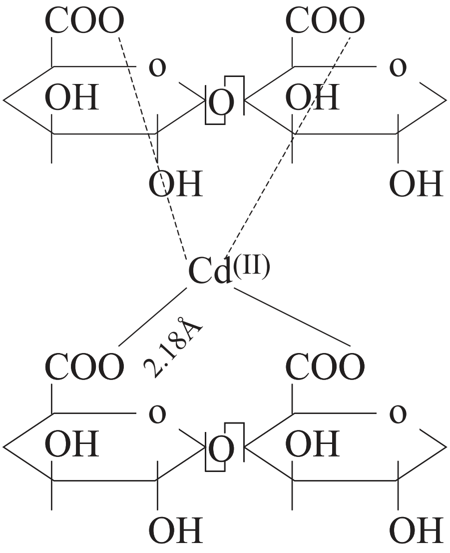


Figure 1.5. Proposed chemical structure for Cadmium sorbed to the surface of dealginated seaweed waste.

The stoichiometry and metal chemical speciation were obtained by ion selective electrode (ISE) and the dissolved concentration by ICP-OES. Dealginated seaweed surface composition and major functional groups were identified by potentiometric titrations and FTIR-ATR. Spatial distribution of metal-biosorbent complexes was obtained by ESEM. Bond distances and coordination chemistry of the structure were elucidated by means of EXAFS. The thermodynamic data produced by this experimental approach can be applied in geochemical speciation models and included in reactive transport models in order to help assess the fate of metals in soil and sediments. An example of existing software that utilizes this approach is the PHREEQC freeware available from the United States Geological Survey (http://water.usgs.gov/software/phreeqc.html). This simulation code contains databases for adsorption reactions, and has the capability for users to additionally define new reactions for simulation of speciation including incorporation into models for reactive transport.

1.3.3. *Implications for risk assessment*

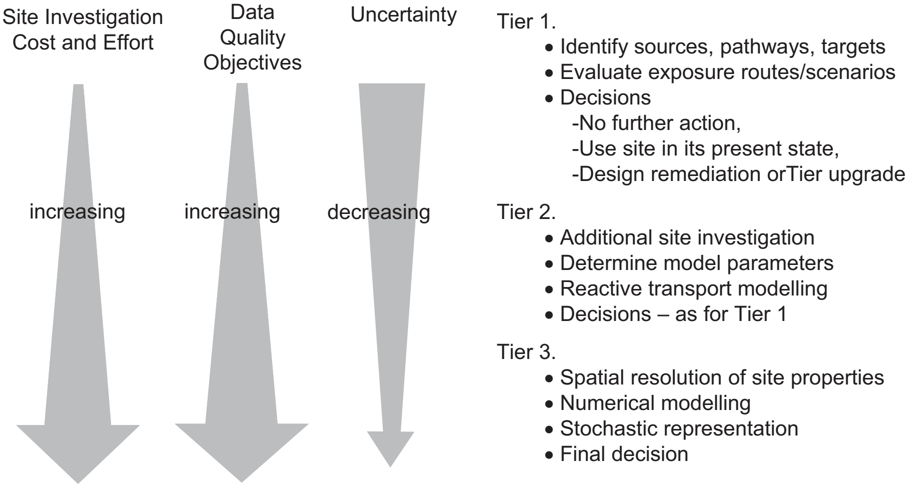
The previous section highlighted the value of characterizing in detail the chemical composition and speciation of the metal and soil or sediment in order to identify and quantify the chemical reactions that are occurring in order to help understand the fate of the contaminant. Such detailed information is necessary to understand the underlying controls of the metal transport and fate in the environment. However, due to the uncertainty that arises from the complexity that exists at field scale, this level of detail is not always directly applicable to risk assessment.

Of crucial importance is understanding sources of uncertainty in an assessment of environmental risk. When making quantitative predictions of metals fate and transport, the types of chemical information described above can help provide important first-order information such as.

1. The degree of variability in the dominant soil properties that control adsorption, and
2. The sensitivity of metals fate (sorption) to these controls.

Such knowledge helps the practitioner understand how error in site data that is used to estimate sorption and metals fate can propagate through the risk assessment procedure. This allows better-informed decisions and more optimal targeting of the site investigation budget for activities that best help to reduce uncertainty.

Figure 1.6 is a diagram of a tiered risk assessment framework that shows how increased data costs are incurred at higher-tiered risk assessment stages, in order to reduce uncertainty. This helps make the point that the most crucial value of detailed chemical models for adsorption is to provide more general conceptual and mathematical models for this important class of reactions. If improved estimates of metals adsorption can be made using general models and databases, with minimal site-specific data, then site investigation costs drop accordingly without a loss of data quality.



Tier 1.
- Identify sources, pathways, targets
- Evaluate exposure routes/scenarios
- Decisions
  - -No further action,
  - -Use site in its present state,
  - -Design remediation orTier upgrade

Tier 2.
- Additional site investigation
- Determine model parameters
- Reactive transport modelling
- Decisions – as for Tier 1

Tier 3.
- Spatial resolution of site properties
- Numerical modelling
- Stochastic representation
- Final decision

Figure 1.6. A diagram of the tiered risk assessment framework.

For the fate of metals in the environment, this has already been achieved with chemical speciation models. Using general thermodynamic models and databases, and using only site-specific data on water composition and temperature, a full chemical model for the distribution of dissolved species can be carried out. Adsorption data for metals on many pure mineral phases is also now largely available in the scientific literature. As more studies are carried out on adsorption on complex organic and mixed-sorbent systems, it is likely that general speciation models will be able to utilize this information. If this is possible, it should allow better estimates of adsorption processes at the level of desk-studies, thus meeting higher data quality objectives for less cost at lower-tiered risk assessment.

As shown in the figure, the best way to reduce uncertainty in risk assessment is by increasing the quality of the data, at a higher cost an effort. However, the amount of effort and costs in developing conceptual models and remediation design is improving the quantitative analysis of the data. The benefit then of developing more general understanding of the processes that produces contamination will contribute to develop general mathematical descriptions, more fundamental data and parameters that are generally applicable to site-specific conditions.

2. Particle and soluble release of organic contaminants from the sediment bed

By definition, sediment contaminants are strongly associated with the solid phase. Thus a first approximation to the transport characteristics of the contamination is the transport behavior of the sediments themselves. This has led to a focus on sediment erosion and deposition as the primary means of contaminant exchange across the sediment-water interface. Typically, however, contaminated sediments have accumulated over time in relatively low energy environments where erosion is not significant or occurs only rarely. The subject of this section is identification of the particle and soluble release processes under such conditions. That such conditions occur is illustrated in Figure 2.1, in which measurements of the vertical profiles of Cs-137, Pb-210 and Be-7 in the sediments of the Anacostia River in Washington DC are shown.

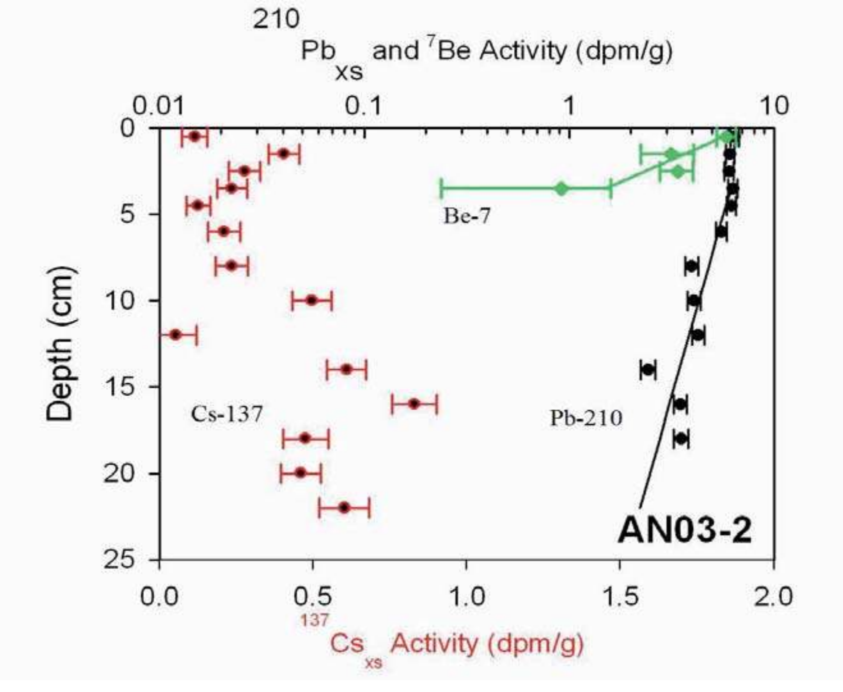


Figure 2.1. Radionuclide profiles in Anacostia River.

The radionuclide profiles shown in Figure 2.1 indicate typical mixing behavior in the surficial sediments and the long term stability of the sediments. Be-7 is a short lived radionuclide and its decrease in the upper 4 cm of sediment implies a rate of mixing of that layer (6 cm/yr). The long term stability is shown by the linear decrease in excess Pb-210 with depth, which suggests a steady rate of deposition without evidence of any erosional events. This is further indicated by Cs-137, in that the presence of this radionuclide indicates sediment at the surface since 1954 when this radionuclide was first introduced to atmosphere by nuclear testing. The release of this radionuclide reached a maximum in 1963, identifying the date associated with the peak in the Cs-137 prfofile. Both the Cs-137 profile and the rate of burial of Pb-210 suggest a steady sediment deposition rate of 0.44-0.66 cm/yr.

In steady depositional situations such as this there is little or no erosion. Because only situations in which there is no net loss of particles are considered, the abiotic release of contaminants across the sediment-water interface is controlled by soluble release. Benthic organisms that live at the sediment-water interface may cause significant reworking of particles within the surficial sediments but are assumed to cause no net release of particles from the bed. Organisms can, however, provide another mechanism for movement across the sediment-water interface by accumulating contaminants within their body mass and introducing these contaminants into the food chain via predation.

Thus, the movement of contaminants into the water column in a stable sediment environment is controlled by the soluble release and by that fraction of contaminants that enter the food chain via accumulation in benthic organisms. The controlling factors and the rate of these processes will be discussed in this section. It will be concluded that both mechanisms are ultimately controlled by the soluble fraction of contaminants and not the bulk sediment concentrations.

2.1. TRANSPORT PROCESSES WITHIN SEDIMENTS

In a stable sediment, that is, one not subject to erosion, there still exist mechanisms for movement of chemicals via pore water processes such as advection and diffusion, as well particle reworking. Of these, any mechanism that causes particle reworking will dominate over pore water processes due to the sorbing nature of most sediment contaminants. Movement of gas or pore water at sufficient rates can move particles but the flows necessary to cause such behavior are not generally observed. Benthic organisms and bioturbation, the mixing process associated with the normal burrowing and feeding activities of these organisms, generally represent the dominant sediment reworking mechanism in sediments. Many of these organisms process sediment directly for food and thus in one feeding cycle may cause movement of chemicals that might require months or years by pore water processes. In freshwater systems, for example, tubificid oligochaetes that are a few cm long and weighing but a few mg, can be found in concentrations approaching 100,000 organisms/m^2 and each may ingest up to 10-20 times their weight in sediment each day. These organisms are generally limited to the upper 10-15 cm of the sediments and their greatest activity is limited to the upper 5 cm. The penetration of Be-7 into the Anacostia River sediments as shown in Figure 2.1 is likely due to the action of these organisms. Thus the Be-7 profile shows both the depth of activity (~4 cm) as well as the rate of activity (~6 cm/yr) resulting from these organisms.

2.2. SOLUBLE RELEASE FROM STABLE SEDIMENTS

In a stable sediment bed, that is, one not subject to net particle loss, contaminant movement into the water column is limited to soluble release. Other processes may rework the surficial sediments and control the rate at which the sediment surface is renewed with contaminants but the rate at which contaminants at this surface move into the water is controlled by soluble processes. Included in soluble release is contamination that is truly dissolved as well as contamination that is associated with colloidal matter that is operationally defined as dissolved. There is an important difference between these two components, however, in that only the truly dissolved fraction is available to directly partition into air, water or organic matter. That fraction associated with colloidal material must normally desorb back to truly dissolved form before partitioning.

Note that the focus on no net loss of sediment still allows short term resuspension of particles, for example by organisms that eject particles forcibly into the water column which settle back quickly to the adjacent sediment surface. In addition, tidal or wave action can cause similar short-term resuspension of sediments that can quickly settle back to the bed surface. The significance of these processes for contaminant release is still dependent upon desorption into the water column and the rate of soluble release during their duration of the particle's exposure to the water column.

The net rate of contaminant release through the sediment water interface can be conceptualized as a process of mass transfer through the upper sediment layers followed by a process of mass transfer through the benthic boundary layer. These two processes operate sequentially and the two-film model can be adapted to describe transport through the sediment-water interface. Conceptually, this approach recognizes that under steady conditions, the flux to the sediment-water interface is equal to the flux away from this interface. Thus the flux, N_A, through the sediment-water interface can be written

$$\frac{N_A}{C_{pw} - C^*} = k_{sw} = \frac{1}{1/k_w + 1/(K_{sw} k_s \rho_s)}$$

Where C_{pw} is the pore water concentration of the contaminant below the sediment surface layer, C^* is the concentration that would be in the pore water if it were in equilibrium with the water (0 if the water were devoid of significant contamination). The mass transfer coefficients, k_w, k_s, and k_{sw}, are defined in the water film, surface sediment layers and overall across the sediment-water interface, respectively. K_{sw} is the effective partition coefficient between the solid phase and the pore water in the sediment. ρ_s is the average density of the particles in the bed.

The above equation can be used to determine the relative importance of the mass transfer resistance posed by the water or sediment side processes. The values of the water-side mass transfer coefficient, k_w, is a relatively well understood function of flow in the overlying water. It is typically of the order of 1 cm/hr and Thibodeaux et al. (2002) determined a range of 12.3-33.3 cm/day for several rivers, including rivers subject to erosion and rivers in which the sediment bed was essentially stable. The value of k_s must be estimated on the basis of measurements of sediment reworking rates and Thibodeaux et al. (2002) estimated a range of 2-3.6 cm/yr for the same rivers. As indicated previously the Be-7 profile in the Anacostia River core referenced earlier was consistent with an effective mass transfer coefficient of 6 cm/yr. Although the numerical value of the coefficient is small, it is based on solid phase concentrations which are considerably higher than pore water or soluble concentrations. The presence of K_{sw} in the above equation is the result of placing the two mass transfer coefficients on the same basis. Assuming a solid phase density of 1 g/cm^3 and a K_{sw} of 10^3-10^5 L/kg, the k_s range of 2-3.6 cm/yr causes the term $K_{sw}k_s\rho_s$ to range between 5.5 cm/day and 1000 cm/day. At the lower end of this range, associated with low sediment reworking rates and low preference for sorption to the solid phase, sediment side mass transfer resistances can control contaminant release. At the higher end of this range, associated with high sediment reworking rates and very hydrophobic and sorbing compounds, the water side mass transfer resistances control contaminant release.

2.3. CONTAMINANT MIGRATION AS A RESULT OF BIOTURBATION

We thus have a picture of the stable sediment-water interface in which the upper layers of the sediment are relatively rapidly mixed and the benthic boundary layer resistance may control mass transfer into the overlying water. The question arises as to the cause of the relatively rapid mixing in the surficial sediment layers. Advective and diffusive processes are operative only on contaminants present in pore water and thus strongly sorbed contaminants are unlikely to be mixed at the observed rates by these processes. Bioturbation, however, the sediment mixing processes associated with the normal burrowing and foraging activities of benthic organisms, can move sediment and associated contaminants at these rates. Deposit feeding tubificid worms, for example, typically are present in densities approaching 100000 m^2 and process 1-5 mg/worm per day in the upper 5 cm of sediment. This is the equivalent of 100-500 g of sediment being reworked per day per m^2 of sediment. Assuming that the sediment has a dry density of 1 g/cm^3, this implies that the upper 5 cm of sediment (50000 cm^3 per m^2) is completely processed in 100-500 days, or an effective reworking rate of 3-15 cm/yr. Thus bioturbation is consistent with the observed rates of reworking of a stable sediment. By comparison, a relatively rapid groundwater seepage rate of 1 cm/day with a compound exhibiting an effective partition coefficient of 1000 L/kg, would only account for a sediment reworking rate of approximately 0.3 cm/yr, smaller than only account for a sediment reworking rate of approximately 0.3 cm/yr, smaller than typical sediment mixing rates. Lower seepage rates would give rise to even lower effective sediment mixing rates.

We thus have an image of a stable sediment as one that is dominated by organism associated sediment reworking within the sediment layer and contaminant migration into the overlying water limited by the rate of sediment reworking and transport through the benthic boundary layer. Bioturbation also has other influences on contaminant fate in the sediments as illustrated by a laboratory study of contaminant depletion with time in a sediment exhibiting an initial uniform concentration of pyrene. The concentration profile initially (broken line) is contrasted with the concentration profile after approximately 3 months with (filled bars) and without (open bars) the presence of benthic organisms (a tubificid oligochaete) is shown in Figure 2.2. Significant depletion of pyrene is observed to extend deeper in the organism populated cells, presumably as a result of deeper oxygen penetration and aerobic degradation of pyrene in those cells.

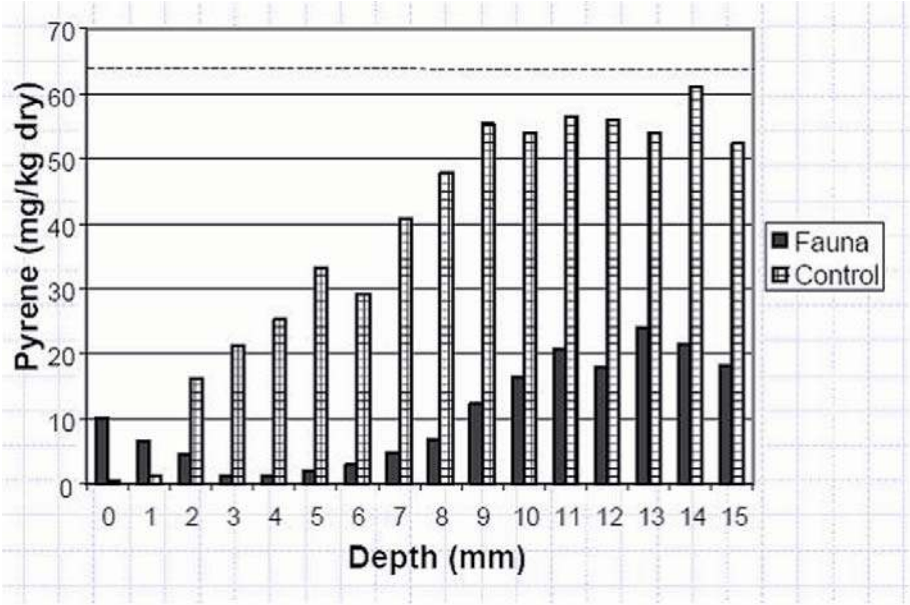


Figure 2.2. Depletion of pyrene in a laboratory microcosm with and without faunal influence (tubificid oligochaetes).

2.4. CONTAMINANT ACCUMULATION IN BENTHIC ORGANISMS

In addition to providing a mechanism for rapid reworking of the surficial sediment and transport of any associated contaminants, benthic organisms also introduce contaminants into the food chain. Organisms ingest or absorb contaminants and these accumulated contaminants can be transferred into the food chain when fish or other predators feed on these organisms. The amount of contaminant that accumulates in these organisms also relates directly to any toxic effects on these organisms. Assessment of contaminant burden that can accumulate in benthic organisms is thus a good indication of the risk associated with the sediment contamination.

Potentially complicating contaminant accumulation in benthic organisms is the strongly sorbed nature of some sediment contaminants. Contaminant sorption or desorption often tends to display biphasic character with a fraction of contaminants readily desorbable and a fraction of contaminants which exhibit desorption that is limited by rate and extent. The question arises as to what extent this desorption resistant fraction of contaminants is available for accumulation in benthic organisms.

This has been studied extensively by Lu et al. (2003) and this work has shown that despite the route of exposure, benthic organisms can ultimately accumulate a contaminant burden that is related to the pore water concentration of contaminant. That is, to the extent that pore water concentrations are reduced by the presence of desorption–resistance phenomena, this would directly reduce steady state accumulation and uptake of contaminants into a benthic organism.

This is illustrated in Figure 2.3, in which the steady state uptake of a variety of polynuclear aromatic hydrocarbons into a tubificid oligochaete is summarized. In every case, the steady state body burden in the organism correlated well with the dissolved phase porewater concentration of the PAH contaminant.

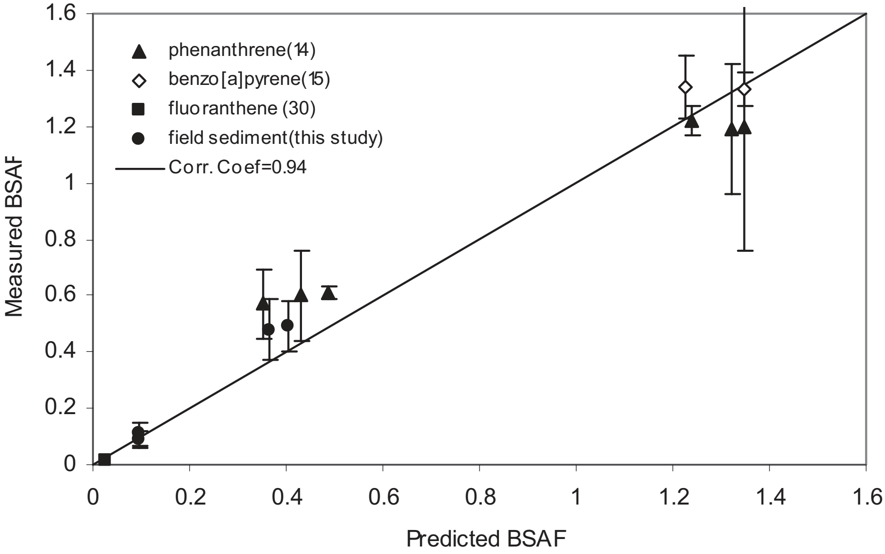


Figure 2.3. Steady state uptake of selected PAHs in tubificid oligochaetes compared to uptake predicted by dissolved phase pore water concentration of the PAHs.

2.5. SUMMARY

This section has demonstrated that the migration and fate of sediment contaminants is often controlled by soluble release. Although sediment contaminants are typically strongly associated with the solid phase, the accumulation of these contaminants is often in locations that are not subject to erosion. In a stable sediment environment, contaminant migration is typically controlled by the reworking of the surface layers of the sediment by the benthic organisms. This process of bioturbation results in rapid movement of contaminants to the sediment surface followed by dissolution and soluble release to the overlying water. In addition, contaminants in this surface layer can be taken up directly by the benthic organisms and introduced into the food chain. Experiments show, however, that even ingestion of strongly hydrophobic contaminants in sediments results in body burdens in the benthic organisms that can be related to the soluble contaminant levels in the pore water.

Thus contaminant movement from sediment into the overlying water can be controlled and defined by soluble release and uptake, at least under conditions such that significant erosion of the sediments does not occur. This soluble component controls or at least indicates both movement into the overlying water by direct release from the sediment bed as well as by introduction into the food chain through accumulation in benthic organisms. Movement within the sediment may be controlled by particle reworking associated with bioturbation but movement into the overlying water in a stable sediment must then be via the soluble fraction.

3. Biogenic influences on sediment stability and rheology: examples from long-term mesocosm study

3.1. INTRODUCTION

The upper few centimeters of aquatic sediments are characterized by extreme gradients in sediment porosity and strength. These gradients exert strong influences on the response of sediments to hydrodynamic and mechanical stresses, and on the exchange of pore fluids and particles (including contaminants) with overlying water, as discussed in Section 2.0. A large body of research suggests that bioturbation by infauna exerts significant influence on shallow-seabed physical properties (e.g., Rhoads and Young, 1970; Rhoads and Boyer, 1982; Richardson et al., 1983; Meadows and Tait, 1989; Jones and Jago, 1993; Rowden et al., 1998) and dissolved and solid-phase chemical transport (Berner, 1980; Boudreau, 1997, and references therein). However, little is known about the feeding and burrowing activities of most benthic macrofauna, and even less is known of how macrofaunal behavior can influence these sediment characteristics. Sediment porosity and shear strength are fundamental properties of the seabed, and understanding biogenically induced variability for these properties is critical to many investigations of seabed stability, sediment dynamics, and contaminant fate and transport. Bioturbation of the seabed has been shown to influence sediment shear strength and erodibility (Rhoads et al., 1978; Dade et al., 1992; Wiberg, 1999), however, both our basic understanding of these influences and our ability to model them remain minimal.

In this study, we examine the temporal and spatial influence of bioturbation on sediment shear strength and porosity using long-term mesocosm observations of a subsurface-deposit feeding enteropneust (*Schizocardium* sp.), and a surface-deposit feeding ophiuroid (*Hemipholis elongata*), placed in compacted, muddy sediment. Specifically, we wanted to know if these organisms are capable creating the vertical porosity and strength distribution typical of modern surficial marine muds (e.g., Dade et al., 1992; Boudreau and Bennett, 1999). The burrowing and feeding styles of these species are typical for two major groups of benthic invertebrates. *Schizocardium* is a funnel-feeding deposit feeder (Fig. 3.1), similar to numerous species of polychaetes, enteropneusts, and holothurians that are distributed from estuarine to bathyal sediments. *Hemipholis elongata* is a surface-deposit feeder that dwells in a simple burrow (Fig. 3.1); many common bivalves and polychaetes have similar lifestyles (see Bromley, 1996, for an excellent review). Both species are common at high population densities (Flint and Kalke, 1986; Valentine, 1991; Rowden et al., 1998), and are probably the most important bioturbators at our collection sites.

3.2. METHODS

Sediment and organisms were collected by box-core from stations in Mississippi Sound (*Schizocardium*: 89° 20.0'N, 30° 14.0'W, 3 m depth; *Hemipholis*: 30° 16.0'N, 88° 46.0'W, 9 m depth). Sediment was sieved at 0.5 mm, placed in glass mesocosm tanks (60 cm wide x 60 cm long x 45 cm deep), and allowed to settle for 3 weeks. Sediment was compacted with ~90 kg of lead to reach a porosity of 0.65-0.7. Sediment for a macrofauna-free mesocosm was frozen to eliminate meiofauna, dewatering the sediment to a porosity of ~0.77. This sediment was not compacted additionally, because it was also intended as a control for other coincident experiments. Mesocosm populations for *Schizocardium* (420 m^{-2}) and *Hemipholis* (300 m^{-2}) were established at mean field densities, and were fed small quantities of fish food biweekly. Salinity (*Schizocardium*: 17 psu; *Hemipholis*: 27 psu) and temperature (19-20° C) were monitored and adjusted twice weekly. Timed fluorescent lamps were illuminated over the tanks for 10 hours per day. Water was filtered through external sumps filled with aragonite gravel.

Burrow morphology and changes in surface-sediment relief were documented by photography throughout the experiment. Surface relief was also assessed during resistivity profiling.

Resistivity was measured using a small, Wenner-type probe (Keller and Frishknecht, 1966). The probe was made of stainless-steel electrodes potted in epoxy resin, with dimensions of 3 x 10 mm, mounted on 3-mm o.d. x 60-cm tube. The signal (100Hz, 1.0 V square wave) was supplied with a Hewlett Packard HP3312A function generator. Voltage from measurement electrodes was measured with a true-rms multimeter. Measurements were made 2 cm above the sediment-water interface, and at 1-cm vertical intervals within the sediment. The probe was calibrated in seawater (17 and 27 psu) prior to each series of measurements. Porosity was estimated using the Archie relationship (e.g., Jackson et al., 1978; Briggs et al., 1998), $FF = R_s/R_f = \phi^{-m}$, where FF is the formation factor, R_s and R_f are resistivity of saturated sediment and overlying water (or measured voltages), respectively, ϕ is porosity, and m is a constant derived from probe calibration, (for this study, $m = 1.69 \pm 0.16$). Porosity estimated from FF was validated against porosity measured by water loss at the start and end of the experiment.

Shear-strength profiles were collected with a hand-held shear vane (4 blades, 1.9 x 1.9 cm diameter x height) attached to a torque-measuring screwdriver (Rhoads and Boyer, 1982; Briggs and Richardson, 1996). Measured torque was corrected for sediment-rod friction (Briggs and Richardson, 1996). Shear strength (τ_f) was calculated from (Holtz and Kovacs, 1981): $\tau_f = (6T_{max})/(7\pi D^3)$, where T_{max} is measured torque, and D is vane height and width. Shear-strength and resistivity profiles were collected at 10-cm intervals along transects located to avoid previous measurements.

3.3. RESULTS

3.3.1. *Biogenic sedimentary features*

Generalized burrow morphology for *Schizocardium* and *Hemipholis* are shown in Figure 3.1. A typical *Schizocardium* burrow consists U-shaped burrow extending >10 cm deep, with a feeding pit (1-3 cm deep) at one opening of the burrow and a fecal mound (1-4 cm tall) at the other (Fig. 3.1), creating total relief of 2-7 cm. As observed through tank walls, the basal portions of burrows are relatively long-lived features (stable for months), whereas the locations of burrow openings are shifted on the order of 1 cm every few days.

A *Hemipholis* burrow is a simple feature, shaped like an inverted bell extending 5-9 cm into the substrate (Fig. 3.1). The central disc of the ophiuroid remains hidden at the base of the burrow, while arms are extended into the water column and across the sediment surface to gather food particles. Occasionally, arms are extended through the main burrow wall, exiting the sediment away from the primary burrow opening. Mounds created by burrow excavation (and possibly feeding activity) produce gentle relief of 1-2 cm on the sediment surface. Burrow locations are not permanent, but the mechanism of burrow relocation (i.e., sideways excavation versus emergence and excavation of an entirely new burrow) is not clear. Experiments documenting more detailed burrow geometry and bioturbation rates for both *Schizocardium* and *Hemipholis* are presently underway.

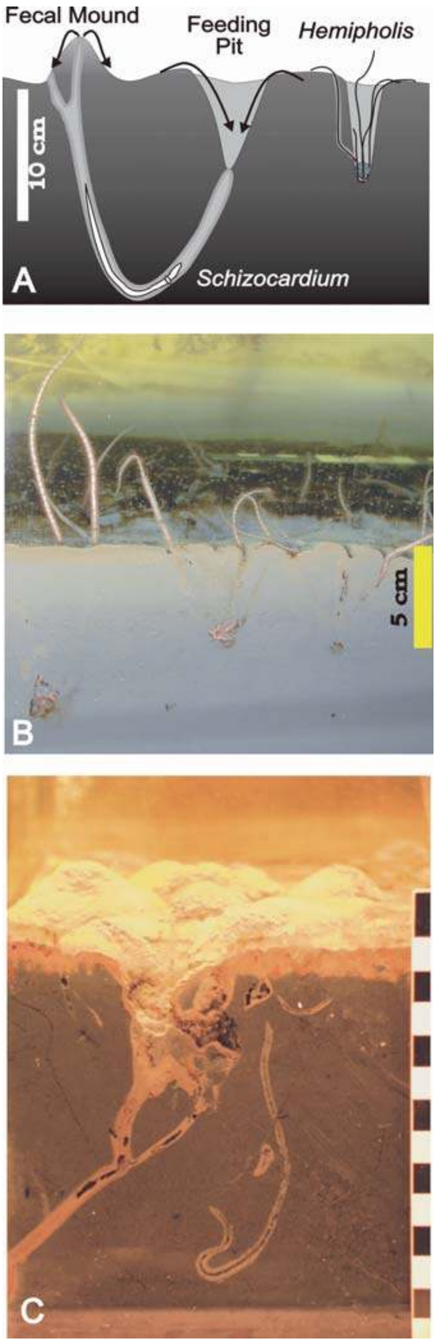


Figure 3.1. Illustrations of *Schizocardium* and *Hemipholis* burrows. (A) Schematic illustration of entire burrow for each organism (scale is approximate). (B) Photo of *Hemipholis* organisms and burrows through aquarium wall. *Hemipholis* arms are frequently extended into the water column, evidently searching for food particles, but are also seen sifting through surficial sediment. (C) Photo of Schizocardium feeding pit through aquarium wall, showing clear geochemical gradients in the sediment. *Schizocardium* appears to ingest both consolidated sediment from walls of the feeding pit, as well as material that falls into the pit. Sediment is egested to the fecal mound as puffs of mucous-laden flocs, with no pelletization or coiled structure.

3.3.2. *Porosity*

The macrofauna-free mesocosm had a vertical initial porosity profile that remained constant for the lower 12 cm of the sediment column throughout the study. However, the upper 2 cm increased in porosity and thickness after day 44, due to inadvertent introduction of meiofauna (very fine burrows were visible in the upper 2-3 cm of sediment). Porosity profiles from Day 98 onward development of a high-porosity layer in the upper 2 cm, with respect to initial conditions (Table 3.1, Fig. 3.2).

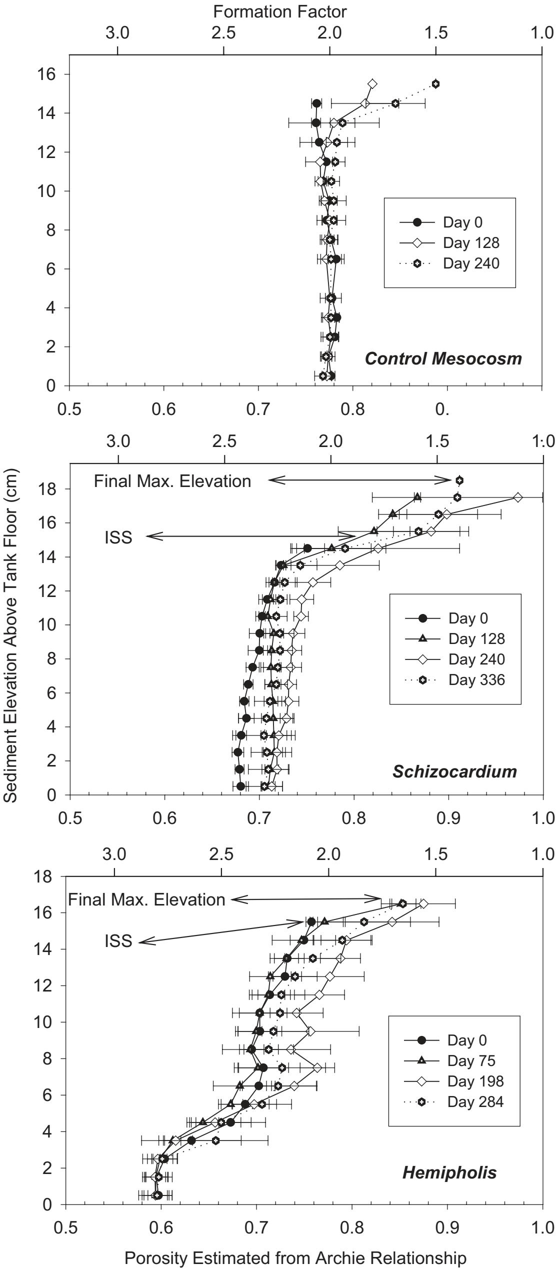


Figure 3.2. Time-series profiles of formation factor and estimated porosity (m=1.69) for experimental mesocosms (mean and standard deviation of 8-11 profiles). Vertical datum is the tank floor, and ISS refers to the sediment-surface elevation at initiation of the experi-ment. In each case, bio-turbation resulted in dilation of some portion of the sediment column, increasing total sediment thickness. The basal gradient in the Hemipholis resistivity profiles results from differential settling of sand during substrate preparation. Sediment from the Hemipholis collection location is ~30:70% sand: mud, whereas sediment used for Schizocardium and macrofauna-free mesocosms is ~10:90% sand: mud.

In the *Schizocardium* mesocosm, the initial nearly vertical profile (ϕ ~ 0.7) evolved to an exponential-type profile with high surface porosity (ϕ > 0.9), and slightly increased basal porosity (ϕ ~0.72)(Fig. 3.2). Sediment thickness increased by ~4 cm, due to the dilation of sediment excavated from feeding pits and ejected onto fecal mounds (Fig. 3.2, Table 3.1). Formation of pits and fecal mounds also increased relief. Dilation (or excavation) was most pronounced in the upper ~7 cm (depth of Z_{50}, Table 3.1), but occurred throughout the sediment column, increasing the volume of the upper 12cm (depth to Z_{90}, Table 3.1) by a factor of ~1.4 during the experiment.

Porosity profiles from the *Hemipholis* mesocosm were strongly influenced by the presence of a basal sandy layer that settled out of suspension during substrate preparation (Fig. 3.2). The formation of a high-porosity surface layer is the most obvious development during the experiment, during which the volume of the upper ~4 cm (depth to Z_{90}, Table 3.1) increased by a factor of ~1.25.

For all mesocosms, sediment inventories indicate close mass balance (and so no measurement artifacts) between initial and final measurements (Table 3.1). However, measurements in the *Schizocardium* (Day 240) and *Hemipholis* (Day 198) tanks at intermediate times produced anomalously high mean porosities and low mass inventories for both tanks (Fig. 3.2). The cause of this is not known, and these data are not used for calculation here.

3.3.3. *Shear Strength*

Shear strength increased by ~0.5 kPa at all depths in the macrofauna-free mesocosm during the experiment, reaching an apparently stable condition after Day 101 (Fig. 3.3). In the both *Schizocardium* and *Hemipholis* mesocosms, shear strength decreased above 9 cm elevation, and increased below 9 cm (Fig. 3.3), but the magnitude of change was greatest in the *Schizocardium* mesocosm. Both populated mesocosms reached apparently stable conditions after 3-4 months of bioturbation (Fig. 3.3).

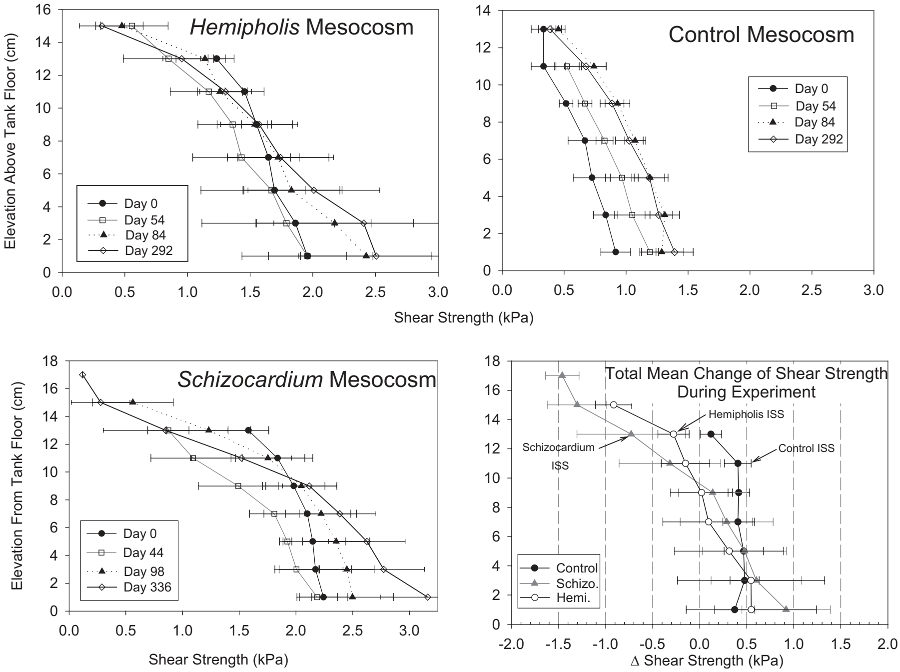


Figure 3.3. Time-series profiles of shear strength in mesocosm profiles (mean and standard deviation of 5-8 profiles). Vertical datum is the tank floor, and ISS refers to the sediment-surface elevation at initiation of the experiment. In each case, macrofaunal bioturbation resulted in sediment weakening with respect to the macrofauna-free sediment, which strengthened during the measurement period.

3.4. DISCUSSION

These results document the biogenic conversion of a relatively firm, homogenous substrate into a very watery, weak, and spatially heterogeneous substrate typical of the muddy seafloor (e.g., Dade et al., 1992; Boudreau and Bennett, 1999). *Schizocardium* is the most effective of the two taxa at modifying both porosity and shear strength, probably because it ingests large volumes of sediment below the sediment surface, egesting the particles as a dilute sediment suspension. (In contrast, *Hemipholis* appears to be a particle-selective surface feeder [Gielazyn et al., 1999; this study]). In the *Schizocardium* mesocosm, the largest changes of shear strength and porosity occur in the upper 6-8 cm of the substrate, although burrows extend much deeper (Fig. 3.1). This probably indicates that *Schizocardium* effects on shear strength and porosity are most closely tied to depth and rate of particle ingestion and egestion (feeding pit and fecal mound formation) rather than burrow depth (Table 3.1, Figs. 3.1–3.3).

Table 3.1. Sediment mass balance and transfer due to bioturbation, estimated from porosity profiles (Fig. 3.2). Vertical datum for comparison is elevation of initial sediment surface (ISS) from tank floor. Particle density of 2.6 g cm-3 was used for calculations.

	Macrofauna-free	Schizocardium	Hemipholis
Duration of Experiment (d)	240	336	281
Sediment Inventory Day 0 (g cm^{-2})	8.25±0.06	11.8±0.09	13.05±0.26
Sediment Inventory at Experiment End (g cm^{-2})	8.20±0.13	11.94±0.35	12.72±0.34
Sediment Mass Lost Below Initial Sediment Surface (ISS) (g cm^{-2})	0.33±0.13	0.93±0.36	0.70±0.43
Mass transferred above ISS (g cm^{-2})	0.29	0.86±0.04	0.38
Maximum Elevation Gained Above ISS (cm)	1	4	1
Z_{50}: Depth Below ISS to 50% of Sediment Loss (cm)	< 1	7	2
Z_{90}: Depth Below ISS to 90% of Sediment Loss (cm)	2	12	4
% Dilation for sediment moved from Z_{50} to sediment surface, based on observed porosities.	129%	272%	189%

The effects of *Hemipholis* bioturbation on shear strength (Fig. 3.3) do not appear to be tied to porosity changes or feeding depth. Because the burrow depth (Fig. 3.1) and depth of shear-strength influence (Fig. 3.3) are similar, this biogenic remolding is probably related to the rate and depth of burrow formation (not feeding) by the *Hemipholis* population, in contrast to *Schizocardium*.

These observations have several important consequences. First, specific biological activities can dilate and weaken sediments in opposition to gravitational consolidation. These opposing processes appear to reach a steady state after 3-4 months. Second, because a functional relationship appears to exist between depth/rate of biogenic activity and depth/intensity of effect, these influences can probably be incorporated into porosity and consolidation models (e.g., Toorman, 1996; Boudreau and Bennett, 1999) in terms similar to those used in diagenetic models (biodiffusion coefficient, mixing/ingestion depth, ingestion rate: Boudreau, 1986; Rice, 1986; Robbins, 1986; Wheatcroft et al., 1990) to portray work done by organisms to create and maintain sediment in a dilated or remolded state.

Future experiments will look at bioturbation rates and depths, and changes in electrical resistivity (porosity/density) and shear strength in initially *unconsolidated* sediment populated by *Schizocardium* and *Hemipholis*. We hypothesize that porosity and strength profiles similar to Figures 3.2 and 3.3 will be established in spite of very different initial conditions. This leads to the prediction of species/sediment specific steady-state profiles of sediment properties in nature.

4. Biogeochemistry of humic substances in a salt marsh estuary

4.1. INTRODUCTION

Salt marshes represent natural ecosystem-level interfaces between terrestrial, fresh and salt water environments. Three major components of this interface are sediment, tidal sea water, and many autochthonous organisms. A major plant component is a vascular plant *Spartina alterniflora* (Loisel.). This smooth cordgrasss densely occupies salt marshes at the East and Gulf Coast of the USA, and is responsible for very high primary production, which amounts approximately 1400 g C m^{-1} y^{-1} (Pomeroy et al., 1981). Although large amounts of different natural and anthropogenic organic and inorganic materials, including pollutants are permanently introduced into salt marshes especially by rivers draining agricultural and forested watersheds, these natural environments remain keeping high productivity and other environmentaly important functions. Apparently, some site-specific components such as humic substances may support these valuable natural properties of a salt marsh estuary (Alberts et al., 1994).

Humic substances have been shown repeatedly to represent a significant fraction of the natural organic matter in both terrestrial and aquatic environments. Also, HS are known to interact with a wide spectrum of inorganic and organic compounds and elements through an array of chemical reaction and physical binding that can influence bioavailability and toxicity of different pollutants (Frimmel et al., 2002; Senesi, 1992). Such a capacity have been repeatedly described for HS in seawater and sediments (Ertel and Hedges, 1983; Harvey and Boran, 1985; Vanderbroucke et al., 1985). In addressing geochemical interactions in the salt marsh, Pellenbarg (1985) examined how *S. alterniflora* litter can interact with waters and sediments, including interaction with some potentially toxic elements.

Since both the tidal water and salt marsh sediment usually consist HS different in concentration and quality, we performed extended studies over years to elucidate the origin and fate of HS in a salt marsh estuary. In our experiments a possible contribution of *S. alterniflora*-plants to the HS-pool was emphasized. It is the purpose of this report to summarize the findings achieved. In its individual sections, the respective references will be indicated.

4.2. *S. ALTERNIFLORA* AS A SOURCE OF HUMIC SUBSTANCES

In a primary study devoted to the topic we investigated whether *S. alterniflora*-biomass consists of constituents which might resemble to HS from a salt marsh sediment (Filip et al., 1988). For this reason, samples of fresh and standing-dead leaves and culms of *S. alterniflora* were extracted under N_2 with 0.1 M NaOH + 0.1 M $Na_4P_2O_7$ mixture (1:1 v/v) for 24 h. The supernatant was acidified with HCl to pH 1 and HS were allowed to precipitate overnight. They were than separated by centrifugation, dialyzed against deionised water and freeze dried. Salt marsh sediment was sampled from a depth of 0-15 cm at the same site as the plants, dried at 60°C, finely ground, and extracted in the same way as the plant material. In Table 4.1 the amounts and chemical composition of HS yielded are presented.

Table 4.1. The yield and elemental composition of humic substances extracted from the biomass of S. alterniflora, and from salt marsh sediment (Filip et al., 1988).

Sample	Humic substances (g dry wt./100g)	Element (ash-free%)			
		C	H	N	O
fresh	0.56	46.0	5.9	5.8	42.3
dead	1.09	52.0	5.6	3.6	33.8
Sediment	2.17	40.4	4.5	2.5	52.2

It was calculated that 0.64% of the C of the fresh plant biomass was extracted as HS, and that in the dead plant biomass, and salt marsh sediment this percentages increased to 1.42 and 9.38 %, respectively. Similarly, Ertel and Hedges (1985) found that humic acids accounted for 1-2% of the carbon from an oak wood. In our experiments, HS were extracted under N_2 in order to minimize a possible autooxidative polymerization of some phenolic plant constituents.

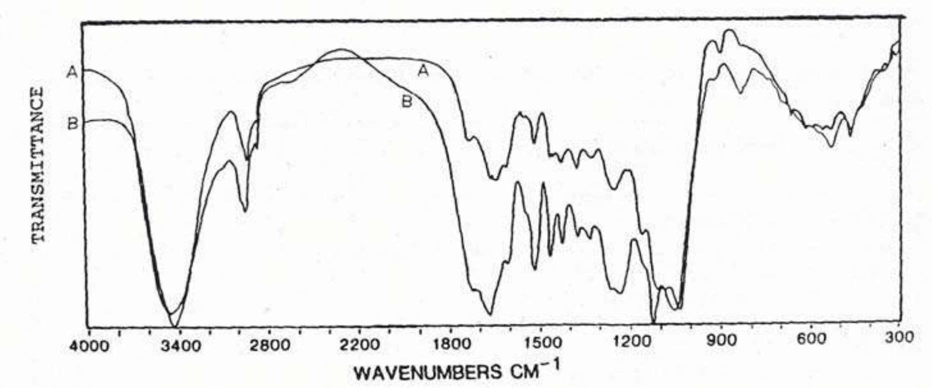


Figure 4.1. FTRI spectra of the S. alterniflora-dead plant biomass (A), and a humic substance extracted from the same material (B). (Filip et al., 1988).

The FTIR spectra (Fig. 4.1) added support to the idea that HS were present in plant tissues before the extraction: Although there are some quantitative differences, almost all the absorption peaks observed in the dead plant-related HS have their counterparts in the spectrum of the parent plant material. The similarity of the individual HS-preparations were evidenced also by UV/VIS spectroscopy. In addition, and irrespective of their origin, the individual HS were shown as concentrating similar amounts of metals such as Cd, Cr, Cu, Mo, Ni, Pb, Sn, Zn. FTIR spectral analyses of different artificially prepared complexes of HS with Ag, Au, Cu, Hg, Mn and Pb suggested that oxygen- and nitrogen-containing functional groups are involved in the metal-binding reactions (Alberts and Filip, 1998).

4.3. MICROBIAL CONTRIBUTION TO THE FORMATION OF S. ALTERNIFLORA-RELATED HUMIC SUBSTANCES

In situ both the green and senescent leaves and stems of *S. alterniflora* are inhabited by numerous microscopic fungi that participate in the decomposition of plant biomass (Gessner and Goos, 1973; Newell, 1984; Leuchtmann and Newell, 1991). Among them, the Deuteromycete *Epicoccum nigrum* was found already earlier to produce dark polymers resembling soil humic substances (Filip et al., 1972). A question arose whether some of the Ascomycetes such as *Phaeosphaeria spartinicola* and *Phaeosphaeria halima* which usually colonize *S. alterniflora* plants are also capable of forming HS-like polymers. Filip and Alberts (1993) performed laboratory experiments of up to one year in duration to elucidate this. They found that fungi did not form dark in color pigments when grown in an artificial, full-nutrient medium. However, if the nutrient solution was enriched with a water extract of *S. alterniflora*-biomass, appreciable amounts of dark colored substances were produced. The FTIR spectra of the respective compounds (Fig. 4.2) demonstrated a high degree of similarity especially between the polymer isolated from the *P. halima*-culture and the HS extracted from *S. alterniflora*-dead plant biomass.

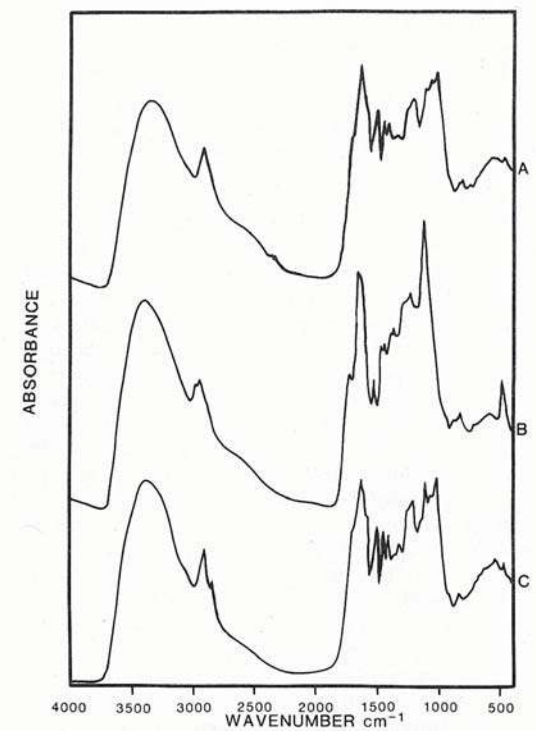


Figure 4.2. FTIR spectra of (A) polymer isolated from culture of P. spartinicola grown in a nutrient solution amended with S. alterniflora extract; (B) polymer isolated from culture of P. Halima grown as under (A); (C) humic substance extracted from S. alterniflora-dead plant biomass (Filip and Alberts, 1993).

4.4. THE RELEASE OF HUMIC SUBSTANCES FROM *S. ALTERNIFLORA* INTO SEAWATER

Different simple in structure organic compounds, e.g., monosaccharides, amino acids, and phenols, have been detected in samples of both fresh and dead *S. alterniflora,* and also in HS related to the respective plant biomass (Alberts et al., 1992). Several authors have found that in part these compounds are leached from the plants into seawater by the rising tide, and may influence carbon metabolism of the autochthonous microbial communities (Fallon and Pfaender, 1876; Gallagher et al., 1976; Pakulski, 1986). In our experiments (Filip and Alberts, 1988), we wished to find out whether, perhaps, also HS from *S. alterniflora* plant biomass can be released into seawater and whether microorganisms indigenous to a salt marsh environment are capable of influencing such a leaching. For this reason equivalent amounts of fresh and dead plant material were incubated in seawater under either sterile conditions or in the presence of different autochthonous microorganisms. Within 10 months the plant material inoculated by *S. alterniflora* epiphytic microbial populations or by a population indigenous to salt-marsh sediment developed a milky skin on the surface, and a dense turbidity in seawater. As shown in Table 4.2, different amounts of HS were obtained from the individual samples of seawater (that was made free of particulate material prior to the isolation of HS).

Table 4.2. Yield of humic substances from the seawater long-term incubated with S. alterniflora plant material added. (Filip and Alberts, 1988).

Sample and inoculum	Humic substances (dry weight) mg l^{-1}	%
Seawater with *S. alterniflora* fresh		
Sterile control	198.7	100.0
Epiphytic microbial population	994.3	500.4
Microbial population from sediment	324.7	163.4
Seawater with *S. alterniflora* dead		
Sterile control	24.2	100.0
Epiphytic microbial population	417.8	1726.4
Microbial population from sediment	301.6	1246.3

The data indicate that the fresh plant material can release up to 820% more HS into seawater than the dead one. However, if related to the sterile control, the effectiveness of the microbial populations was much greater with the dead plant material. Nevertheless, both fresh and dead biomass of *S. alterniflora* is apparently capable of contributing not only some simple organic compounds but also considerable amounts of HS to the pool of dissolved organic carbon in seawater. In addition, the remaining fresh and dead plant material appeared another possible source of HS to the salt marsh environment. If extracted by alkali (see in Paragraph 1) appreciable amounts of HS could be additionally obtained (Filip and Alberts, 1989). According data published by Moran and Hodson (Moran and Hodson, 1990) up to 20% of the total dissolved organic matter appears in the form of HS on the southeastern USA continental shelf.

4.5. MICROBIAL UTILIZATION OF SALT MARSH-RELATED HUMIC SUBSTANCES

A principal question in biogeochemical studies concerns the stability of organic substances in a diagenetic environment. According to Rashid (1985), humification, i.e. formation of HS, represents the major process in early diagenesis. Thus, it should be recognized that *S. alterniflora* plants represent an important factor in diagenetic processes occurring in a salt marsh estuary. Usually, HS are considered as refractory organic matter (Frimmel et al., 2002), and accordingly, salt marsh-related HS were considered as little degradable by microbial activities (Pomeroy and Imberger, 1981; Fogel et al., 1989; Esham et al., 2000).

In a specific experimental approach, we wished to established, whether *S. alterniflora*-related HS, and those extracted from salt marsh sediment can resist to attack by microorganisms indigenous to that environment (Filip and Alberts, 1994). The HS were used as additional sources of nutrients or as single sources of either C or N in a nutrient broth. The individual preparations were incubated for 15 days (aerobic cultures) or 12 months (semi-anaerobic cultures) with a complex population of salt marsh sediment-related microorganisms. Depending on cultivation conditions and type of HS, between 27% and 100% of the added HS were utilized. The highest utilization rates were obtained with HS related to *S. alterniflora* fresh and used as the sole source of N, but also sediment-related HS served as efficient nitrogen sources in aerobic cultures (78.1% utilized). The remaining HS-preparations recovered from aerobic cultures exhibited elemental and structural changes that are characteristic for diagenetic transformations of sedimentary HS: Increase in C content, C:H and C:N ratios, and IR absorption typical for aromatics. The slopes of the spectra in the visible range of light indicate an increasing optical density, and thus somewhat higher degree of "structural condensation" for the HS preparations re-isolated from microbial cultures (Fig. 4.3). Under semi-anaerobic cultural conditions the diagenetic transformations of HS did not occur in the same way as in the aerobic cultures. Rather, within a year the HS from *S. alterniflora* fresh was completely utilized by microorganisms. Esham et al. (2000) found a bacterial community from seawater capable of mineralizing only up to 3% of HS obtained from a decomposing *S. alterniflora* biomass. However, these authors used only indirect methods such as CO_2 measurements and a loss of light absorbance as an index of HS-degradation which may have influenced final results as recognized earlier (Filip et al., 1998).

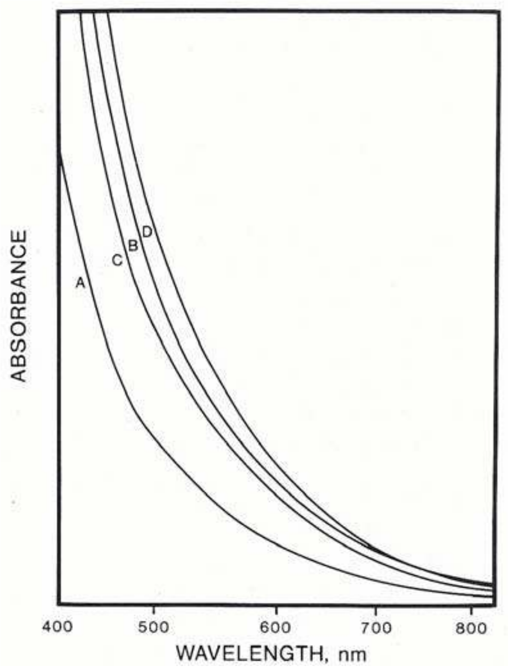


Figure 4.3. Visible spectra of humic substances (A) extracted from salt-marsh sediment; (B) the same substances incubated for 15 days under aerobic conditions in a full-strength nutrient broth; (C) the same substances incubated as a sole source of carbon; (D) the same substances incubated as a sole source of nitrogen (Filip and Alberts, 1994).

4.6. ADSORPTION AND TRANSFORMATION OF HUMIC SUBSTANCES BY QUARTZ AND CLAY MINERALS

The adsorption of organic material dissolved in seawater by suspended mineral particles may prove to be a very significant process in removal of such organic matter from water and of incorporating it into the bottom deposits. Concerning salt marsh-related HS, one can assume that their adsorption on sedimentary particles may play a role in supporting their protection from biological decomposition, and thus making them geochemically more stable.

Rivers deliver large quantities of mineral materials into the estuaries, from which about 30% are clay minerals, particularly montmorillonite and kaolinite (Bader et al., 1960). In addition, the tidally-influenced water bodies receive an important influx of clays from shorline erosion and bottom scouring. Botom sediments of Sapelo Sound (which was our main experimental site) contain a clay fraction composed of 30-45% kaolinite, and 50-60% of expandable clays (Pinet and Morgan, 1979). Some other salt marsh sediments were found to contain up to 82% quartz sand (Bradley and Morris, 1999). Therefore, we considered it important to investigate whether HS related to salt marshes are capable of forming stable complexes with some clay minerals and quartz, and whether their elemental and structural characteristics change as a result of adsorption on these minerals (Filip and Alberts, 1994). The amounts of salt marsh-related HS which were adsorbed on quartz, kaolinite and montmorillonite are shown in Table 4.3.

Table 4.3. Adsorption of salt marsh-related humic substances (HS) on quartz and clay minerals (HS in mg TOC l^{-1}) (Filip and Alberts, 1994).

Source of HS	Orig.HS	HS ads. on quartz		HS ads. on kaolinit		HS ads. on montm.	
		TOC	%	TOC	%	TOC	%
S. alternifl. fresh	95.8	11.3	11.8	77.3	80.6	80.7	84.2
S. alternifl. dead	89.9	9.9	11.0	72.1	80.2	71.8	79.9
Salt marsh sed.	98.4	9.3	9.4	83.2	84.6	88.5	89.9

The rate of adsorption did not differ substantially for the individual HS preparations. The lowest amounts of HS were adsorbed on quartz but still, they comprised up to 11% of HS added. Surprisingly, the amounts of HS adsorbed on montmorillonite were similar to those adsorbed on kaolinite. This indicates that very little interlayer adsorption apparently occurred on montmorillotite. The C contents generally increased in HS which remained in solution after mineral-bound HS-fractions were removed by centrifugation. The differences were higher after adsorption on kaolinite and ranged from 13.5% for HS from *S. alterniflora* fresh, to 7.9% for HS from *S. alterniflora* dead, and 4.3% for those from sediment. These features indicate a preferential adsorption of low in C structural components of HS, i.e., some aliphatic structural units.

The FTIR spectra shown in Fig. 4.4 indicate, that no significant change in the structure of *S. alterniflora*-fresh related HS occurred due to the adsorption on quartz. The HS remaining in the solution after a portion was bound on kaolinite and montmorillonite show more differences in comparison to the original preparations. Among the most striking features observed was the decrease in intensity of the aliphatic C–H stretching at

2925 cm^{-1}, a shift of carbonyl C–O stretch in amide I band at 1654 cm^{-1} to an aromatic C═C stretch at 1615 cm^{-1}, and loses in intensity of the amide II and amide II bands at 1517 and 1230 cm^{-1}. Since the absorption at 3340 cm^{-1} which can be attributed to H-bonded OH groups and partially to NH groups also lost intensity, one can assume that aliphatic chains such as in alkanes and some primary and secondary amides with their carbonyls, and further carboxyl and OH groups are involved in the HS-clay interactions which occurred in our experimental system. An increase in aromaticity of HS remaining after interaction with montmorillonite was indicated by a strong absorption band at 803 cm^{-1} which accounts for C–H bonding absorption of substituted benzoic acid (Bellamy, 1975). Structural changes of HS are likely to have ecological consequences by modifying the solubility of contaminants, biotransformation processes, and the carbon cycle in soils and sediments (Myeni et al., 1999).

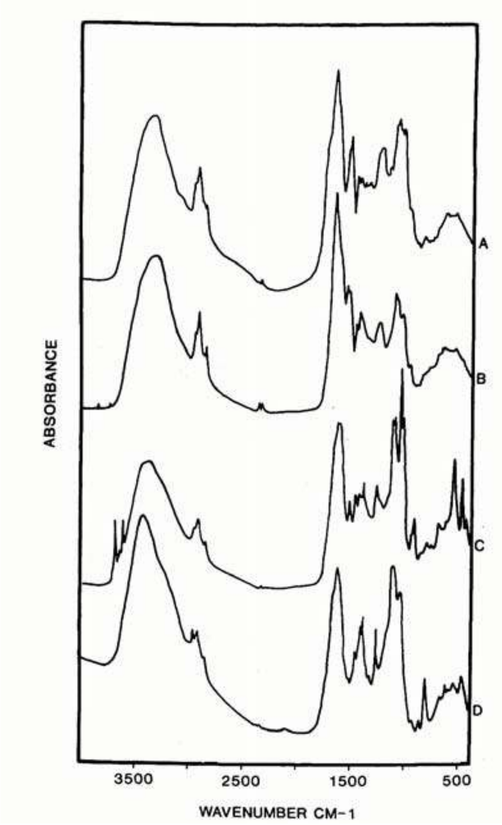


Figure 4.4. FTIR spectra of humic substances (HS) isolated from S. alterniflora-fresh biomass; (B) HS-fraction remaining in solution after adsorption on quartz; (C) HS-fraction remaining in solution after adsorption on kaolinite; (D) HS-fraction remaining in solution after adsorption on montmorillonite (Filip and Alberts, 1994).

An attempt was made to remove the adsorbed HS from the complexes with minerals by acidic or alcalic extraction. When using 0.1 N H_2SO_4, up to 20.2% of HS bound on quartz but only up to 6.7% of HS bound on montmorillonite were recovered. The desorption was more complete when using a mixture of 0.1 M NaOH + 0.1 M $Na_4P_2O_7$. A maximum yield of 41.9% of salt marsh sediment-related HS was extracted from quartz-HS complexes. Other maximum yields were 48% for HS from *S. alterniflora*-dead adsorbed on kaolinite and 68.4% for the same HS adsorbed on montmorillonite. All HS re-extracted from kaolinite or montmorillonite contained a large proportion of ash (between 50.2 – 88.7%) which was prevalently composed of the respective clay minerals as evidenced by FTIR spectroscopy (not shown). This typical feature and the fact that only some amounts of HS could be re-extracted from complexes with minerals indicate appreciable amounts of HS as to be transformed in a fraction of humins, i.e., organic-mineral complexes that are known for their long-term stability in both aquatic and terrestrial environments (Hatcher et al., 1985).

4.7. CONCLUSIONS

Using the annual primary production data (approx. 1400 g C m^{-2} y^{-1}) for the temperate salt marshes and the fact that 85% of that production is in above-ground *S. alterniflora* biomass (Pomeroy et al., 1981), it may be estimated that about 66 g C m^{-2} y^{-1} are associated with HS related to *S. alternilora* fresh material (Alberts et al., 1988). These HS comprise mainly of photosynthetically bound bioelements (plant nutrients) and energy, and apparently exist in the original plant material of *S. alterniflora*. On the other hand, they also consist of polymeric substances produced from water soluble components of the plant biomass by different epiphytic fungi. Due to the activity of complex populations of microorganisms indigenous to salt marsh environments, these HS can be released into seawater and salt marsh sediment. Many evidences exist on the similarity between sedimentary HS and those ones related to *S. alterniflora* fresh or dead plant biomass. Complex microbial populations indigenous to salt marsh sediment are capable of utilizing HS as additional sources of nutrients, and even more intensely as sole sources of carbon or nitrogen. In the presence of quartz particles or clay minerals typically occurring in salt marsh sediments, HS form organic-mineral complexes which in part can resist to the solubilization by acids or alkali.

According to Malcolm (1990), a uniqueness of humic substances is a typical feature in soil, stream and marine environments. Due to their specific origin, chemical composition and structure, and their diverse fate, salt marsh related HS may represent an example of the uniqueness of such substances in a salt marsh estuary. The formation, degradation, transformation, and complexation processes of these HS account for an important part of the biogeochemical cycling of organic matter in a salt marsh ecosystem.

5. Scaling contaminant distributions and contaminant processes

5.1. BACKGROUND

Risk assessment is an integral component of decision making in contaminated sediment management, and an important driving force is the presence of uncertainty. Uncertainties accumulate at all stages of site assessment and modeling and are magnified during scaling of cause-and-effect relationships from laboratory to field scale, from site scale to regional scale, and during extrapolation of relationships beyond their range of input measurements or beyond the site where the relationships/models were established. Even when preventive measures are taken by following sound planning, sampling (Batley, 2002) and analysis methods to eliminate and minimize some sources of error and bias, uncertainties always remain. Nevertheless, the element of uncertainty in the management of contaminated sediments is often treated inadequately (Suter, 1999) or not addressed (e.g. “Critical Issues for Contaminated Sediment Management”, Apitz et al., 2002). For example, unquantified uncertainties can force extremely conservative estimates (Linkov et al., 2002), which can lead to high inefficiencies in the allocation of sparse resources for remediation. Most frequently, uncertainty is expressed in the form of confidence intervals, or by incorporation of safety factors based on best “professional judgment”, such as a factor of 1.5 suggested by Palermo et al. (2002) in the design of cap thickness for contaminated sediments. But these measures and strategies incorporate assumptions about the nature (such as normal distributions) and magnitude of uncertainty, which are rarely tested and which vary along with sampling and measurement methods, models, and site variability and other site characteristics.

Several approaches are available for system modeling and decision-making under uncertainty, depending on the data, the magnitude and the acceptable level of uncertainty. Weight-of-evidence (WOE) approaches deal with large uncertainties by integrating multiple lines of evidence into a decision measure using methods ranging from best professional judgment to weighted scoring and statistical summaries/comparisons to reference situations (Chapman et al., 2002). WOE is most often applied in risk assessment, but its methods are valuable in any situation where uncertainties are large and/or multiple lines of evidence for the same decision requirement are available. When systems are better understood and mechanistic models available, the inputs can be treated as random variables. Uncertainties of these inputs (parameters/coefficients and variables) can be expressed in the form of probability distributions (using regression, analytical error parameters, or best professional judgment in the context of Bayesian prior and posterior probabilities). These uncertainties are then propagated to the model output via mathematical analysis (e.g. ISO/GUM) for simple models and via stochastic simulation: Monte Carlo analysis, Latin hypercube sampling, and bootstrap simulation (Kleijnen, 1997).

The distribution of input *parameters* must often rely on professional judgment, in particular when values of parameters are scattered in the literature and were derived in different experiments (or sites), by different investigators, under different experimental (or site) conditions (Linkov et al., 2002; Steinberg et al., 1997). When experiments are repeated, (non-) linear regression of a functional relationship will yield regression estimates and standard errors used as parameter estimators in appropriate distributions, e.g. the Gaussian distribution (Goovaerts et al., 2001). In the case of *variables* (i.e. field data), probability distributions can be generated using, for example, the known precision of the instrument. If the sampling is spatially distributed, local distributions at all sampled and unsampled locations can be derived using kriging methods. Once input distributions are quantified, values for each input variable and model parameter are randomly drawn from their probability distributions using, for example, a Monte Carlo approach, preserving correlations among the inputs if applicable (e.g. Figure 5.1).

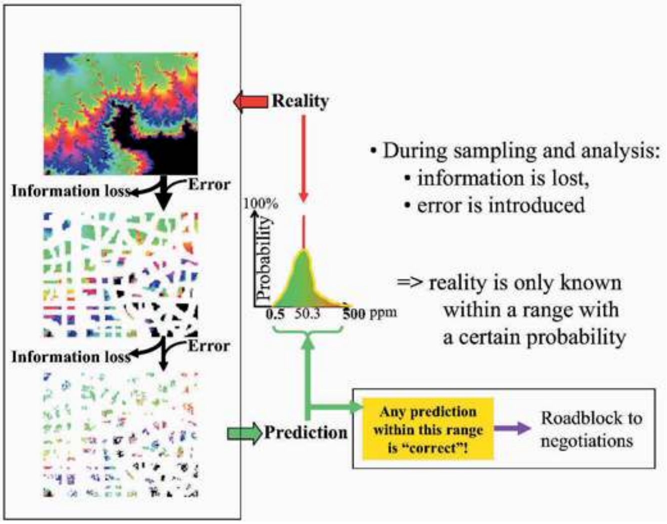


Figure 5.1. Principles of uncertainty imparted by information loss during point sampling, and the need for probability distribution functions to make uncertainty-based predictions.

The resulting combination of input values are used in the model (e.g. sediment fate and transport, contaminant flux) to generate a realization (Papadopoulos and Yeung, 2001; Clarke and McFarland, 1999; Rossi et al., 1993). The process is repeated, while the nature of the sampling ensures that the realizations are equally probable, allowing a statistical summary of the model outputs. Uncertainties associated with scaling (e.g. laboratory derived contaminant degradation used in site models) or aggregation can be assessed in the context of validation, weight-of-evidence approaches (Steinberg et al., 1997), and by gathering additional data that represent the larger-scale patterns of variability (Loxmoore et al., 1991; Barabas et al., 2003ab).

The applications of geostatistical techniques to spatial analysis of sediments are recent and few. Barabás et al. (2001) modeled spatial uncertainty in dioxin contamination in three dimensions using indicator kriging in Passaic River sediments, Murray et al. (2002) mapped the extent of DDE contaminated sediment thickness on the Palos Verdes shelf using sequential indicator simulation, while Fioole et al. (1998) developed the SURFIS computer program, to integrate geostatistical methods in order to account for random error in the optimization of dredging of contaminated sediments with digital terrain models. The basis of geostatistics is the semivariogram, which is a mathematical expression of dissimilarity (e.g. of concentrations) as a function of separation distance between any two points in space (Goovaerts, 1997). The geostatistical approach models an attribute z at each unsampled location $\mathbf{u}$ as a random variable $Z(\mathbf{u})$ with a ccdf $F(\mathbf{u}; z|(n))$, conditional to n neighboring data $z(u_a)$. The ccdf fully captures the uncertainty at $\mathbf{u}$ since it gives the probability that the unknown is no greater than any given threshold z (Goovaerts, 1997): $F(\mathbf{u}; z|(n)) = \text{Prob}\ \{Z(\mathbf{u}) \leq z|(n)\}$. Parametric and non-parametric methods exist to model attribute ccdfs. Indicator kriging (IK) is a non-parametric approach, with the advantages that the shape of the ccdfs is derived using spatially varying information rather than assuming a global analytical expression (such as a parametric Gaussian model). IK is also able to integrate these with uncertainties due to analytical methods (Saito and Goovaerts, 2002), as well as to incorporate additional information on correlated secondary attributes and soft information (such as soil maps, or qualitative field observations) into the modeling procedure. Least squares interpolation algorithms such as kriging tend to smooth out local details of the spatial variation of the attribute with small values typically overestimated and large values underestimated. To avoid these errors, geostatistical stochastic simulation is increasingly preferred to kriging for environmental assessment applications such as delineation of contaminated areas (Debarats, 1996; Goovaerts, 1997b; Kyriakidis, 1997; Naber et al., 1997), or the modeling of groundwater flow in heterogeneous aquifers (Dagan, 1982; Desbarats and Srivastava, 1991; Kitanidis, 1997; Morgan et al., 1993; Desbarats, 1996; McKinney and Lin, 1996).

The set of alternative realizations generated provides a quantitative measure of spatial uncertainty. Features that appear consistently on most of the simulated maps are deemed certain, as expressed by a corresponding local conditional cumulative distribution function (ccdf) summarizing the probability distribution of the attribute at each simulated grid point. The uncertainty can then be summarized into probability maps, risk maps, and maps of false positives and false negatives (Goovaerts, 1999). Such results can aid in a more scientifically grounded assessment of safety factors and a more realistic and comprehensive formulation of confidence intervals. These maps are also well suited to the communication of uncertainties to non-scientific communities.

5.2. METHODS

The statistical premise of the M-Scale model is that the variation of values at different spatial scales is heuristically related. In other words, if samples are collected at the "point scale" (e.g. single cores selected from large sites), the variation of parameters collected within the core are propagated to higher scales by taking into account the correlations of the local averages between scales, as shown in Figure 5.2. These correlations are informed by the variation of local averages at each scale. Taken together, the contributions of variation at each scale are weighted and aggregated into a best linear unbiased estimator, as shown in Figure 5.2c.

The model not only serves as a tool to evaluate parameter relationships over different scales by their covariances and data uncertainty, but also makes further use of these covariances and data uncertainty as the basis for a precision-optimized estimator. Information from each scale is weighted by the projected similarity to the scales of interest, with adjustments incorporating the different precision they provide. Unlike conventional geostatistic tools that are based on point-to-point spatial structures, the multi-scale model introduces a new framework for spatial analysis in which regional values at different scales are anchored by the correlations of each other.

Both normally distributed and bi-modal datasets, representing environmental contamination and microbial impact on contamination through dechlorination were evaluated. These datasets include: 1. A 396 sample characterization in three dimensions of dioxin contamination in the Passaic River (NJ), with emphasis on 2,3,7,8-tetra-chloro-dibenzo-p-dioxin (TCDD), previously mapped using geostatistical techniques (Barabas et al., 2001, Figure 5.3), and 2. A derived map of microbial dechlorination signatures of dioxins in the sample locations (Barabas et al., 2004ab). These signatures were obtained using polytopic vector analysis (PVA), a multivariate statistical technique which allows for unmixing of a complex mixture of contaminants in its weighted source contributions.

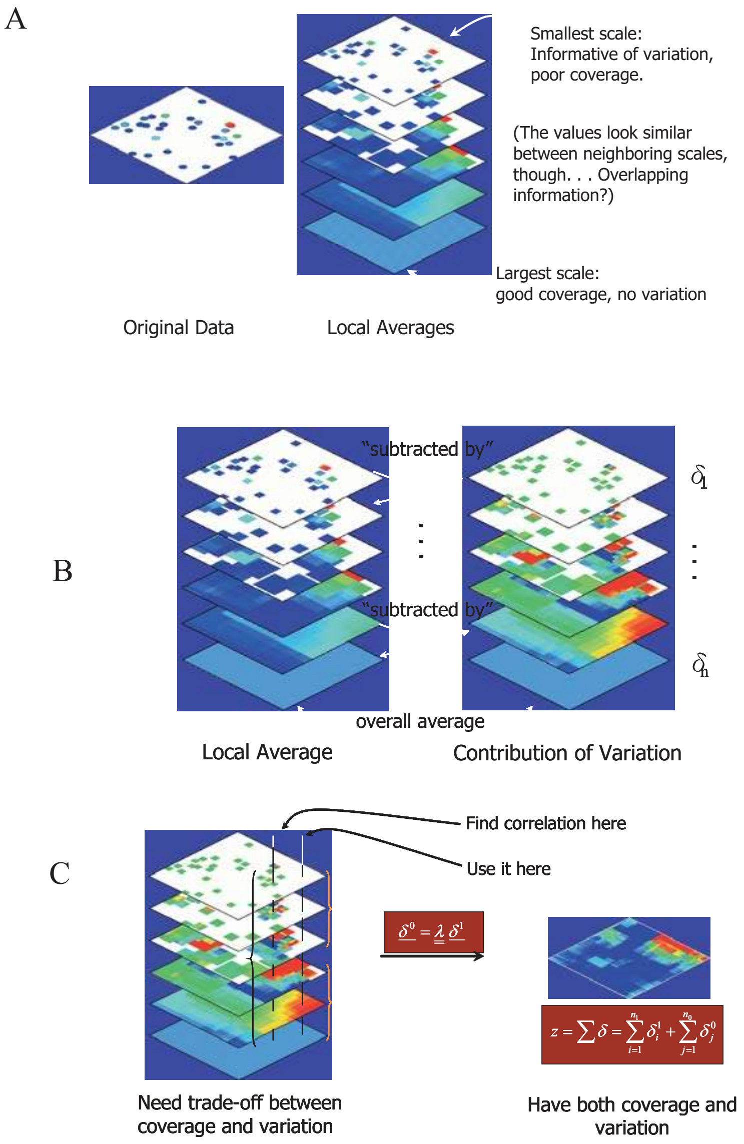


Figure 5.2. Schematic components of the M-Scale model: A. Integration of average values at different scales; B. Determination of variances between scales; C. Correlation between scales at sampled locations to estimate variation at each scale.

Both datasets were analyzed using ordinary kriging and M-Scale, and the quantile-quantile plots mapped to compare both methods for their predictive capability. To ensure robustness of the comparison, both cross-validation (removal and re-estimation of one sample point at a time) and bootstrapping (removal and re-estimation of multiple datapoints) approaches were employed. Only cross-validation data will be shown.

5.3. RESULTS AND DISCUSSION

The Passaic River has been contaminated with dioxins from a wide range of point and diffuse sources of industrial and urban (combined sewage overflows) origin. Multiple datasets in xyz-direction have been collected, and the 1996 dataset is presented. The histogram indicates that the data are log-normally distributed. The process data, based on microbial fingerprints observed in the samples, explaining up to 7% of the pattern variance, were bi-modally distributed. For brevity of this document, these data will not be presented here; the dioxin distribution information should be viewed as illustrative of the results gained from M-Scale and OK analysis.

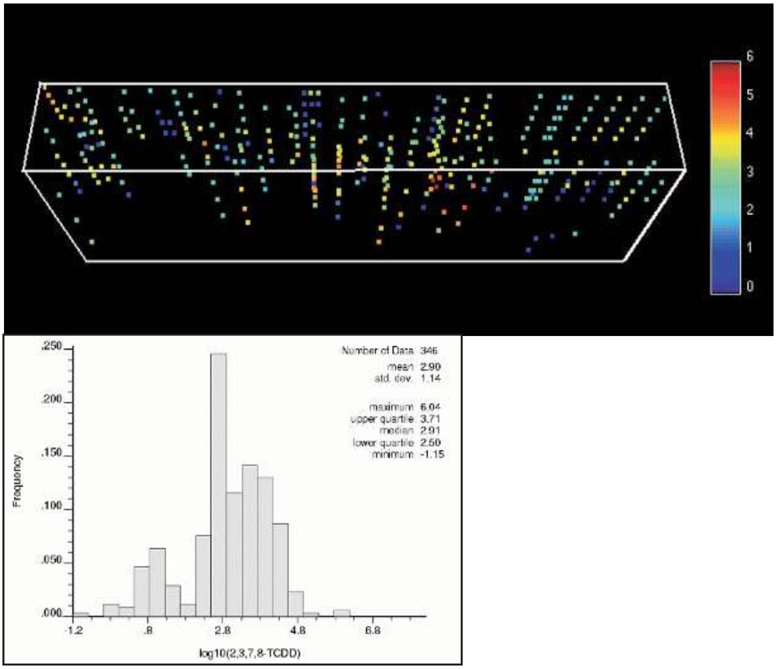


Figure 5.3. Original dioxin distribution and data histogram for 396 TCDD measurements.

A comparison of Ordinary Kriging estimate (left) with M-Scale estimation (right) for two selected cross-sections of the river (Fig. 5.4) indicates that M-Scale appears to preserve local features in contamination, and that OK smoothens out the concentration distributions.

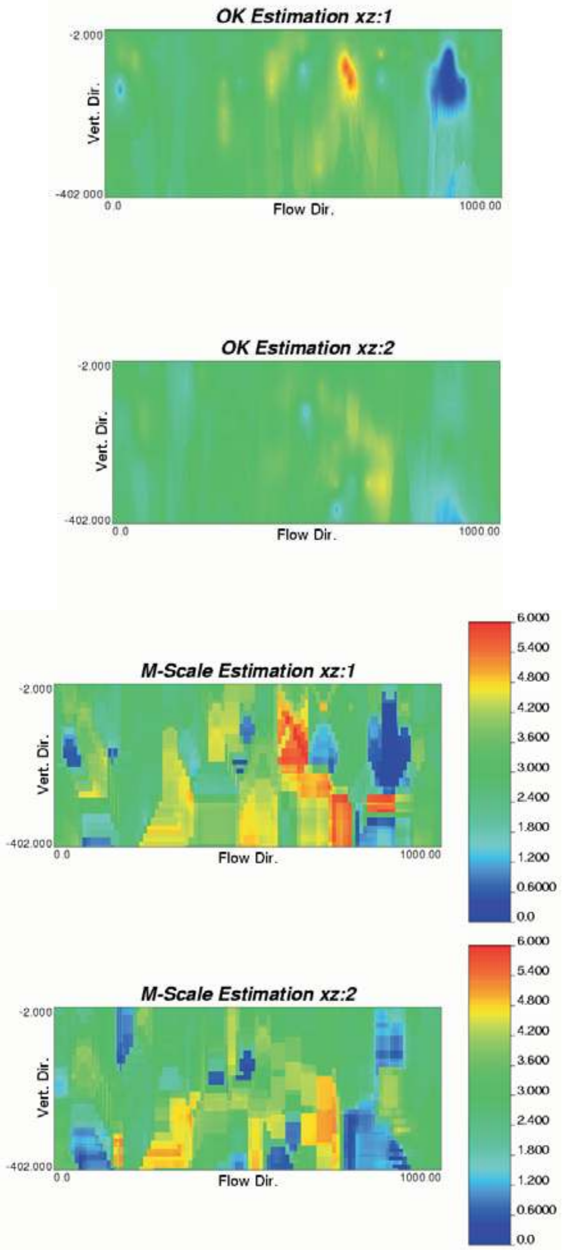


Figure 5.4. Estimation Maps by Ordinary Kriging (OK) and M-Scale Models.

When developing a new estimator for spatially-interpolated data, it is important to report the estimation uncertainty as well. Figure 5.5 (computed for transect 1), shows a correlation between sample density and reduction of uncertainty, as the lower left quandrant in Figure 5.5 exhibits higher uncertainty. The estimation uncertainty map was similar for the OK-based data interpolation (not shown).

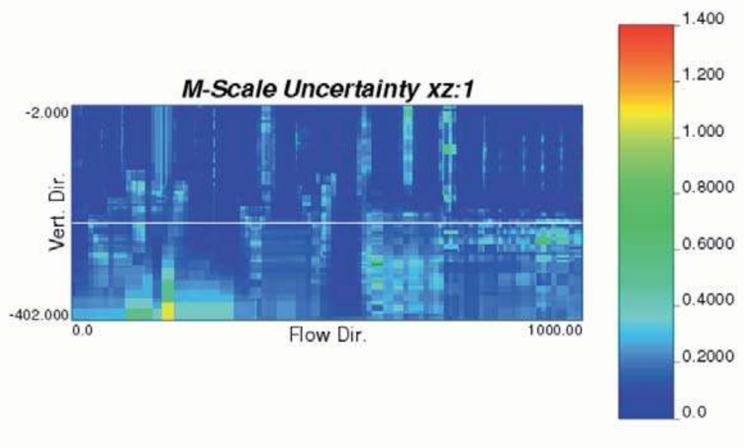


Figure 5.5. Spatial distribution of estimation uncertainty for M-Scale.

The Q-Q plot of the estimated cross-validation results (Figure 5.6) represents graphically how well the model estimates predict the actual values (deviation from the 90° line). The results further confirm that the M-Scale estimation generally reflects the data across the entire magnitude range, while Ordinary Kriging tends to underestimate higher values and overestimate lower values.

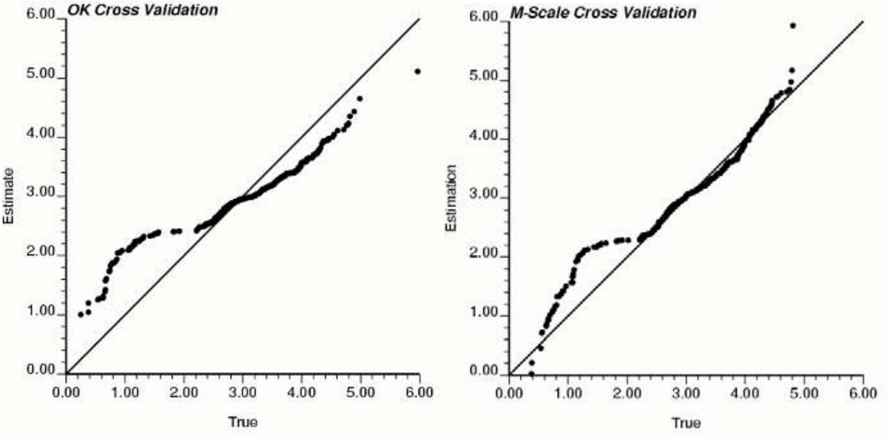


Figure 5.6. Q-Q Plots from Ordinary Kriging (OK) and M-Scale Models.

5.4. CONCLUSIONS

The currently developed methodology for spatial interpolation represents an innovative approach to include multiple scales to estimate attributes in unsampled locations. This capability is of considerable use to inform risk-based decision-making, as the approach allows for the preservation of the overall value distribution (i.e. good coverage of information across scales). The applications, demonstrated here for contaminant distribution as part of site characterization, also include estimation of processes (e.g. biodegradation reactions, microbial respiratory activity, ebullition fluxes, etc…) in environmental systems. Its particular appeal is apparent for the opportunity to quantify spatial uncertainty associated with *in situ* implementation of remediation technologies, as the accuracy of value distributions allows for technology scaling from pilot demonstration. The method development requires further validation against variogram-based spatial uncertainty estimation for different spatial data structures and histograms (value distribution) to demonstrate robustness and application limitations.

6. Appraisal of the problem: what are the gaps in our understanding?

Through consideration of the preceding studies and their implications, we have identified two major types of uncertainty in our understanding of contaminated sediment assessment and characterization. The first we refer to as causal uncertainty, which refers to the difficulty in conclusively demonstrating that a specific observation is the result of a specific process operating at a particular rate (such as the role in macrofaunal biodiffusion in controlling fair-weather pollutant concentrations in a riverine water column). The second type we refer to as statistical uncertainty associated with quantitative predictions of pollutant distribution, fate, and transport, based on either statistical or computational process models. We feel that need for new understanding in these two areas should drive new research and development in contaminated sediment characterization and assessment.

New research, development, and demonstration efforts should focus on issues where reduction in causal and/or statistical uncertainty would make a significant difference in the decision-making process. With respect to contaminant assessment, fate, and transport, both causal and statistical uncertainty exist in the following general areas: (a) process understanding (e.g., measurement and prediction of critical parameters and rates for individual processes such as sediment erosion, consolidation, or biogenic mixing); (b) relationships and interactions among processes (for example, the influence of bioturbation on sediment erodibility or sediment-water contaminant transfer); and (c), forecasting, modeling, and integrating tools (for example, development of transient computational models to predict sediment dynamics, biogenic mixing, and contaminant transfer between the sediment bed and water column). Following is a brief summary of research needs in this area.

6.1. SPECIFIC AREAS OF UNCERTAINTY

- There are no standard or universally accepted approaches for measuring or predicting critical shear stresses and erosion rates for cohesive sediments, including the evaluation of biogenic effects on these sediment properties.
- The dynamics of pollutant binding with humic substances (free and mineral association) and the dynamics of pollutant adsorption/-desorbtion to/from humic organomineral complexes are not well understood.
- No standard approach exists for quantification of spatial (xyz) microbial attributes using surrogate parameters.
- Present *in situ* validated correlations for microbial activity are not adequate.
- Temporal trends of chemical distributions and microbial impact on their fate are not well understood.

6.2. SPECIFIC RESEARCH NEEDS

These specific needs are not limited to topics discussed in this chapter. All measurement and predictive tools should provide estimates of statistical uncertainty.

- Develop, evaluate, and validate standard tools and techniques to reduce uncertainty in measurement and prediction of critical shear stress, erosion rate, mode of transport, depth of physical reworking, and depositional processes for cohesive sediment, including biogenic effects on critical shear stress and erosion rates.
- Develop, evaluate, and validate a standardized approach to propagating uncertainty and representing sensitivity of the processes and interactions controlling sediment stability and contaminant transfer.
- Develop, validate, and distribute standard computational transient fate and transport models that incorporate the above approaches for characterizing sediment dynamics and contaminant mobility, including macrofaunal biogenic effects.

- Evaluate estuarine production and cycling of humics, with a focus on the role of free humics and mineral-associated humics on pollutant fate and availability.
- Develop understanding of how sediment biogeochemical sediment composition influences contaminant partitioning and bioavailability.
- Quantify exchange processes with overlying water and groundwater.
- Investigate impact of sparse sampling and variable histograms/spatial distribution on model robustness
- Evaluate approaches for data fusion (incorporation of additional information, such as inexpensive proxy estimates for more difficult/expensive analyses) on predictive reliability
- Investigate optimization schemes based on trading of scale with minimization of risk classification (i.e. what are misclassification rates as a function of probability thresholds)
- Develop robust approaches to deal with outlying data points.

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TREATMENT AND CONTAINMENT OF CONTAMINATED SEDIMENTS

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Abstract- Several approaches to the containment and treatment of contaminated sediment were evaluated, including the efficacy of adding activated carbon to sediment as an in-situ stabilization method and the use

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of conventional and innovative treatment caps. The applicability of phytoremediation for dredged sediments and sediments in shallow water or wetlands was also explored. The effectiveness of any treatment relies on successful application in the field and the emphasis herein is on evaluation of these approaches in the field. A demonstration at Hunters Point, San Francisco Bay, California strives to prove the efficacy of activated carbon treatment, and stands as an example for applications elsewhere. Simultaneous containment and treatment of sediment contaminants is being demonstrated in the Anacostia River, Washington, DC using both organic and metal sequestering agents incorporated into a cap. Both conventional placement and placement in a laminated mat were demonstrated to provide an array of placement approaches. The laminated mat allowed placement of thin layers of high value material at specific location, enabling the use of high value "active" capping materials such as activated carbon and microscale iron. Phyto- and rhizoremediation using plants and related bacteria is a promising approach for treatment of contaminated sediments, but has its inborn limitations. To overcome the slow performance of the process, transgenic plants were evaluated that express the bacterial gene responsible for cleaving PCBs, or metal binding proteins to provide additional metal binding capacity. Continued development of cap and sediment treatments, and approaches to assess effectiveness and long-term reliability are encouraged.

Keywords: capping, activated carbon, reactive core mat, bioavailability, phytoremediation, transgenic plants

1. In-situ treatment of contaminated sediments by activated carbon amendment

1.1. THE LIMITATIONS OF CURRENT APPROACHES FOR REMEDIATION OF ORGANIC CONTAMINANTS IN SEDIMENTS

Hydrophobic organic pollutants, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and dichlorodiphenyltrichloroethane (DDT) are well-known for their toxicity to humans and wildlife, wide geographic distribution, and slow degradation. Once deposited in water, these chemicals bind with organic matter and, via sediment association, provide a continuous source of such contamination to water and biota. Numerous remedial strategies are available for management of contaminated sediments, each with their own limitations. Dredging is the prevalent ex-situ approach of sediment remediation, which destroys benthic habitat, may incompletely remove contaminated sediments, and may resuspend contaminated fines that migrate to surrounding waters. Further, the disposal of contaminated dredged sediment presents significant problems, both technical and political. The current alternative to dredging is capping of sediments. This method relies exclusively on adequate application and long-term structural integrity, and therefore may prove problematic over time. Mandates against filling productive wetlands also exist in areas such as San Francisco Bay that make capping inappropriate.

Such limitations of remediation alternatives materialized at Lauritzen Channel in Richmond, CA, an EPA Superfund site. The sediment is highly contaminated with DDT, a chemical that causes death and sterility of birds and fish, and possible carcinogenic and endocrinal effects in humans. The channel is an active industrial waterway near a marina and the Rosie the Riveter National Historic Park. Before remediation, resident mussels contained the highest levels of DDT and dieldrin measured in California (Kohn and Evans, 2004). The selected sediment remediation remedy prescribed in the Record of Decision in October 1995 specified dredging and dewatering of all soft bay mud material with off-site disposal and placement of clean material (sand) cap to enhance habitat value (USEPA, 1996). Disposal of the sediments was planned to occur in Colorado Springs, Colorado, but public outcry moved the location to Mobile, Arizona. After dredging was completed in 1997, the sediments were hauled by train to Arizona, but growing public protests in Arizona eventually diverted some of the sediment to a site in Utah (Bruggers, 1997).

Other problems with dredging were evident at Lauritzen Channel even after finding an adequate disposal site. Post-dredging marine monitoring of water and fauna by Pacific Northwest National Laboratory (PNNL) found residual DDT contamination levels exceeding by nearly 200 fold the water remediation goal of 0.59 ng/L (Antrim and Kohn, 2000). Sediment cores sampled by us in 2003 exhibited high DDT concentrations (250 mg/kg sediment maximum) and a stratification of contamination. Some cores showed the remnants of the applied sand layer overlain with highly contaminated sediment. These data suggest the sand layer was not evenly distributed or washed away, and that contaminated sediment remains above and below the applied sand layer. Several biological studies with Lauritzen sediment following dredging found remaining toxicity to amphipods, with several sampling stations rated among the most toxic of coastal sites measured nationwide (Anderson et al., 2000; Weston et al., 2002). The remedial action therefore proved ineffective, and the remaining contamination poses a risk to biota and human health. Currently, the

California Department of Toxic Substances Control advises against eating fish from the Richmond Harbor area, and the EPA and Army Corps of Engineers (ACE) are studying alternatives for the next remedial action.

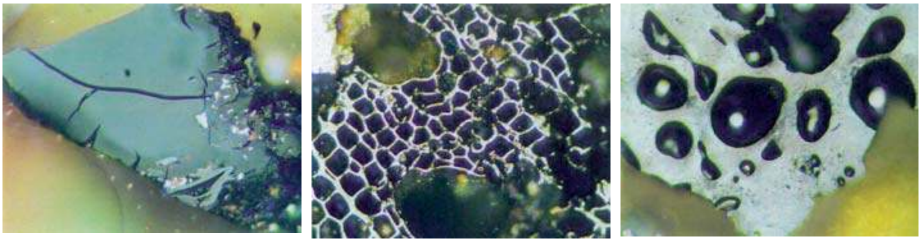
As illustrated by the efforts at Lauritzen Channel, an easy solution for remediating contaminated sediments does not exist. Possible solutions lie in the non-removal treatments of contaminated sediments, such as reactive caps, monitored natural recovery, and in-situ stabilization. Each of these alternative sediment management approaches has its own advantages and is currently being evaluated at sites in the United States. The remainder of this section will focus on the use of in-situ stabilization for PCB-contaminated sediment at Hunters Point Naval Shipyard, San Francisco Bay, CA.

1.2. IN-SITU STABILIZATION: PROOF OF CONCEPT AT HUNTERS POINT

As dredging methods prove ineffective to deal with contaminated sediments, recent research at Stanford University has tested an in-situ alternative to achieve the ideals of high containment effectiveness, limited taxing of the ecosystem, and low costs. The alternative is based on in-situ stabilization with activated carbon amendment. This concept was formulated from the field observation that PCBs naturally, over time, accumulate on black carbonaceous particles in the sediment at Hunters Point. We propose that by mixing activated carbon into the biologically active upper layer of sediment, PCBs will repartition and be sequestered in the carbon, thus reducing PCB bioavailability and release to water. The path forward for advancing this new treatment relies on relating sediment chemistry and effects, on both the micro-scale level of contaminant binding and the macro-scale level of bio-uptake, modeling carbon dose and time effects, transitioning the technique to the field, and engaging regulators and advisory groups.

1.3. DISTRIBUTION OF PCBS AND ADSORPTION EFFICIENCY

Hunters Point is an inter-tidal zone in the South Basin of San Francisco Bay historically contaminated with PCBs (0.2-65 mg/kg total PCBs). In a micro-scale study of the sediment, carbonaceous particles such as coal, coke, char and charcoal (see Figure 1.1) contributed 5-7% of total mass and held about two-thirds of the PCBs (Ghosh et al., 2003). This and related work (Ghosh et al., 2001) showed that compared to other types of particles, PCBs and PAHs accumulate to a much greater extent in black carbonaceous particles and become more strongly bound and less bioavailable. The black carbonaceous particles comprise a portion of the total organic carbon that



oxidized coal charcoal coke

Figure 1.1. Petrographic identification of organic carbon particles in sediment. Images shown are under reflected light in oil at 500x magnification.(Ghosh et al., 2003).

strongly affects the partitioning of PCBs and PAHs, because of its high surface area and adsorption affinity.

Bioavailability, as measured by the absorption efficiency of 2,2′,5,5′-tetrachlorobiphenyl (PCB-52) by clams (*Macoma balthica*), varies among the different carbonaceous particles (McLeod et al., 2004). The absorption efficiency is the highest from wood and diatoms and the lowest from activated carbon, about 1.4% (Figure 1.2). This shows that PCBs bound to activated carbon are not readily absorbed even if ingested.

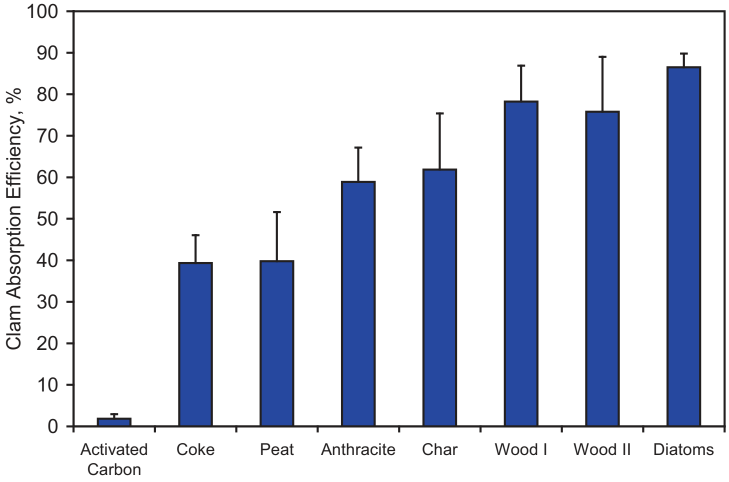


Figure 1.2. Absorption efficiency of PCB-52 varies significantly among different particle types. Error bars represent 95% confidence intervals.(McLeod et al., 2004).

1.4. LABORATORY FEASIBILITY TESTING

After initial findings pointed to the effectiveness of in-situ stabilization with activated carbon, several laboratory feasibility tests were performed with Hunters Point sediment. These tests investigated how activated carbon affected PCB bioaccumulation by several benthic organisms, physico-chemical system properties, and sediment erosion.

Bioaccumulation studies were designed to measure PCB accumulation in three benthic marine organisms plus survival, growth, reproduction, and activity. In the studies, activated carbon was mixed with sediment from Hunters Point for one or six months. After mixing, clams (*Macoma balthica*), polychaetes (*Neanthes arenaceodentata*), and amphipods *(Leptocheirus plumulosus*), were placed in the sediment, and bioaccumulation and biological effects were evaluated in 28 or 56 day tests (Millward et al., 2005; Zimmerman et al., 2005). Results were compared to those obtained using untreated Hunters Point sediment.

A significant decrease in the PCB bioaccumulation as compared to the untreated sediment was noted in all organisms. In 56 day exposure tests, reductions of 90% and 93% were measured for the amphipod and worm, respectively. A clear dose-response was also noted for these organisms in the 56 day test (Figure 1.3). PCB body burden significantly decreased as the dose of activated carbon increased. Similar trends were noted for the clam.

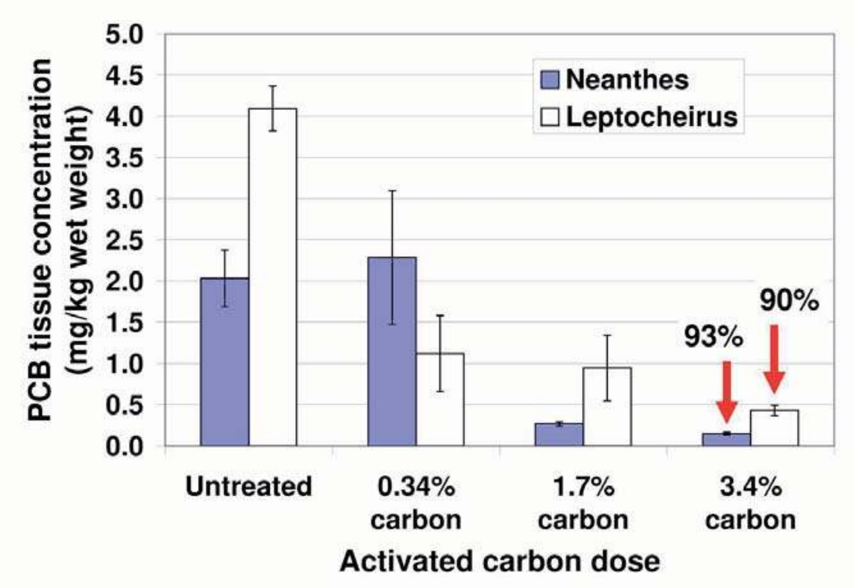


Figure 1.3. PCB accumulation by *Neanthes arenaceodentata* and *Leptocheirus plumulosus* during 56-day exposure tests. Sediment was contacted for one month with varying doses of TOG carbon before tests (Zimmerman et al., 2005).

Exposure to activated carbon amended sediment resulted in no significant effect on the survival, growth, lipid content, and fecundity in the amphipod or survival and lipid content in the polychaete. The polychaete's wet weight did decrease by approximately 50%, but direct toxicity was discounted as an explanation for the effect. All these tests point to the effectiveness of activated to carbon to sequester PCBs from sediment, thus reducing PCB bioavailability.

The positive bioaccumulation tests results were mirrored in physicochemical tests performed in a laboratory. After Hunters Point sediment was contacted with activated carbon in well-mixed systems, the aqueous equilibrium concentration was tested. Reductions up to 92%, as compared to untreated sediment, were measured for a six-month contact time (Zimmerman et al., 2004). As with bioaccumulation, a clear dose response was observed for aqueous equilibrium concentrations (Figure 1.4). In addition, more efficient reduction was noted for lesser chlorinated PCBs. Thus, the repartitioning of PCBs from sediment to activated carbon may be faster for the lower chlorinated and less hydrophobic congeners. The differences observed for aqueous equilibrium concentrations among PCB homologues may be a matter of slower mass transfer kinetics.

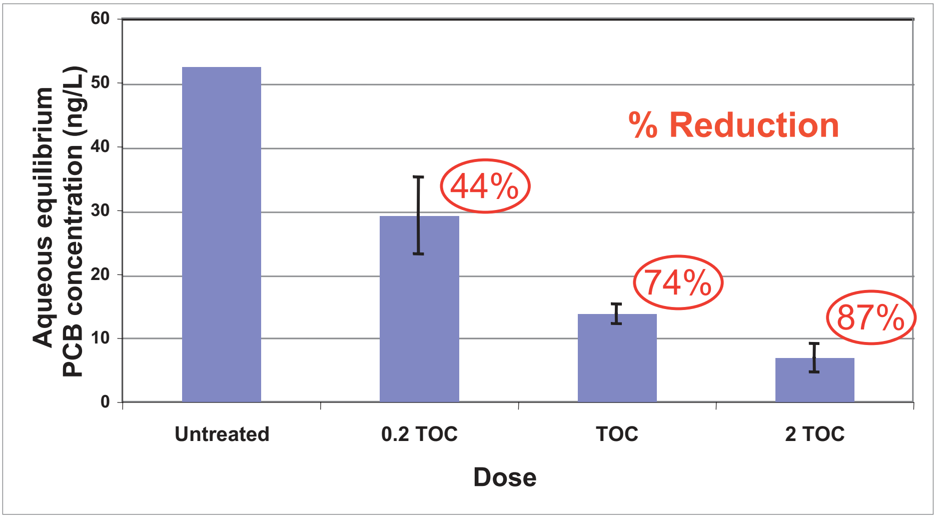


Figure 1.4. Aqueous PCB concentrations for Hunters Point sediment contacted with varying doses of activated carbon for one month (Zimmerman et al., 2005).

Uptake of PCBs by semi-permeable membrane devices (SPMDs), biomimetic devices for lipid uptake in fatty tissue, and measurement of quiescent flux also followed the same dose response to activated carbon treatment. These tests utilized sediment mixed with activated carbon for 1 month. The uptake and flux were reduced with increasing activated carbon doses. Maximum reductions of 73% and 81% were obtained for SPMD uptake and quiescent flux, respectively (Zimmerman et al., 2005).

As shown in both physicochemical and bioaccumulation tests, increasing the carbon dose increases treatment effectiveness. Increased reductions in aqueous equilibrium concentration, SPMD uptake, quiescent flux, and bioaccumulation by a worm, amphipod and clam were all noted with increasing dose. Similar effectiveness in reducing aqueous equilibrium concentration and SPMD uptake was measured for a freshwater PCB-contaminated sediment obtained from a rural site at Lake Hartwell, SC (Werner et al., 2005) and in ongoing tests at the DDT-contaminated industrial site of Lauritzen Channel, CA.

In addition to assessing the effectiveness of activated carbon for in-situ stabilization, issues related to how this technique might be applied in the field are important considerations. To investigate if activated carbon mixed into sediment would stay in place at Hunters Point, a Sedflume device was used to measure the critical shear and stress needed to erode sediment. Results indicated that the sediment at Hunters Point is cohesive, the activated carbon is retained in the sediment under normal tidal conditions, and the critical shear stress is not decreased by mixing carbon into the sediment. Collectively, these laboratory tests suggest that the use of activated carbon stabilization of PCB-contaminated sediment in the field is feasible in terms of effectiveness of treatment and application.

1.5. FIELD DEMONSTRATION

Laboratory-scale bioaccumulation and physicochemical testing provide an important weight of evidence for the efficacy of the proposed activated carbon treatment. However, field demonstration is necessary to evaluate the efficacy and limitations of such innovative treatment technique. Field testing at Hunters Point started in the summer of 2004 and will continue through 2008 (Battelle et al., 2004; Cho et al., 2005). Challenges in the field include effective carbon deployment, minimization of sediment resuspension and mobilization, and adequate field monitoring tools. To meet these challenges, the field approach contains four goals, delineated in the following sections.

1.5.1. *Demonstrate and compare effectiveness of two large-scale activated carbon mixing technologies*

The cohesive sediment hinders the use of heavy equipment on the mudflats at Hunters Point. To combat this challenge, large-scale mixers were identified that either float to position during high tide or could effectively mix from a barge on the shoreline. The Aquamog (Aquatic Environments, Concord, CA), is a large barge-like craft with a rotovator attachment. The craft moves into position during high-tide, and at low tide settles onto the sediment. The rotovator would mix activated carbon applied on the surface into the sediment. As an alternative, the slurry injection system (Compass Environmental Services Inc., Stone Mountain, GA), comprises a mountable base and a long metal arm ending in prongs containing injectors/mixers. Through these mixers, a slurry of activated carbon can be injected and mixed into the sediment. Testing of both mixing technologies will not only show the effectiveness of use at Hunters Point, but also provide important information for use at other contaminated sites.

The effectiveness of the two mixing technologies will be compared by measuring that uniformity of activated carbon mixing and the effects to the indigenous community structure. Five plots will be used to assess success: 1. control, 2. Aquamog mixed, no activated carbon addition, 3. slurry injection system mixed, no activated carbon addition, 4. Aquamog activated carbon application, 5. slurry injection system activated carbon application. Application success will be measured by taking cores in all five plots before and after treatment. The amount of homogenous mixing and any detrimental effects on the biological community will then be quantified. Initial assessment trials showed well-mixed activated carbon resulting from surface carbon application and mixing by the Aquamog (Battelle et al., 2004; Cho et al., 2005).

1.5.2. *Demonstrate reduced PCB availability*

Bioaccumulation and physicochemical testing will be used to measure the success of stabilization of PCBs in Hunters Point sediment during field treatments. Sediment collected before and after mixing will be used to measure reductions in SPMD uptake, aqueous equilibrium concentrations, and sediment PCB desorption rates. PCB bioaccumulation in field-deployed clams (*Macoma nasuta*) and resident amphipods (*Corophium* spp.) will be examined once before and twice after treatment. These data in combination with laboratory feasibility testing mentioned previously should provide an engineering evaluation of the efficacy for field application of PCB availability reduction using activated carbon in-situ stabilization.

1.5.3. *Evaluate possible sediment resuspension and PCB release*

During field activated carbon application, sediment resuspension and PCB release should be minimized to ensure that contamination does not spread to adjacent areas. Before and after activated carbon application, suspended solids and dissolved PCBs will be measured over each test plot. The effect of mixing on suspension can then be quantified, and changes made in application protocols if necessary. Preliminary assessment trials measured no resuspension of PCBs in the water column after mixing of carbon with the Aquamog.

1.5.4. *Coordinate and gain approvals from regulatory agencies*

Hunters Point, as many contaminated areas, is a Superfund site, and activities are regulated by several agencies including the EPA, the Navy, and the California Water Quality Control Board. Any work at Hunters Point, therefore, requires a large amount of coordination among different groups to obtain an approved field test plan (Battelle et al., 2004). In order to achieve success as field trials continue, the coordination with agencies, public interest groups, and other consultants working at the site must also continue.

1.6. CONCLUSIONS

Current remediation techniques for management of contaminated sediment have significant limitations, including those of dredging for which disposal is a significant problem and residual contamination leads to continued contaminate availability to water and biota, and therefore humans. In new research, however, a look at the indigenous components of sediment that sequester hydrophobic organic contaminants has led to intense investigation of the efficacy of adding activated carbon to sediment as an in-situ stabilization method. Numerous laboratory feasibility studies found increasing the dose of activated carbon mixed with contaminated sediment strongly affects the degree of reduction in PCB availability. Similar work shows the possibility of use at other sites contaminated with hydrophobic chemicals. The effectiveness of any treatment, however, relies on successful application in the field. Success is quantified by measurements of mixing, reduced PCB availability, and minimal resuspension. Engaging regulators early in the treatment evaluation process helps ensure the development of a successful testing program and allows for ease of transition to the field.

The continuing work at Hunters Point strives to prove the efficacy of treatment, and stands as an example for applications elsewhere. As work continues, the long-term stability and fate of PCBs, application to others sites and contaminants, and possible activated carbon retrieval after deployment will be studied. The work thus far is a case showing how being outcome-oriented during feasibility testing and engaging regulators early can allow for a timely transition of a new technology to the field.

2. Cap and treat technologies

Sediment contaminants such as polychlorinated biphenyls (PCBs) and heavy metals are a widespread and costly environmental problem. Source controls can reduce the input of contaminants into ecosystem, but the slow and continual release of sediment contaminants to the benthic community can significantly degrade a riverine or estuarine ecosystem. Human exposure to PCBs is believed to be predominantly through the food chain (trophic transfer) via fish consumption (Thomann and Connolly, 1984). In situ capping (ISC) can be a useful tool for managing contaminated sediments and improving the overall quality of the aquatic habitat, and ISC overcome some of the problems associated with dredging (e.g. residuals and ex-situ storage and treatment costs) and monitored natural attenuation (e.g. insufficient rate of burial). ISC eliminates contact between the benthic community and the underlying contaminated sediment with a physical barrier of clean material, usually sand. The objectives of ISC are to isolate and stabilize the underlying contaminated sediment, reduce contaminant flux into the biologically active portion of the sediment, and create new habitats for aquatic organisms (Reible et al., 2003). Layered sediment cap designs have been proposed (Palermo et al., 1998), which incorporate active layers to treat porewater transporting through the cap (Figure 2.1). This active layer may consist of sorbents to retard PCB porewater transport or media that provides biotic or abiotic dechlorination. Active layers in the sediment cap provide the ability to simultaneously isolate PCBs from the benthic community for long times and provide PCB mass removal.

2.1. CAP COMPONENTS

Sediment capping with sand or clean sediment has been used to improve sediment stability and isolate contaminants for many decades (Talbert et al., 2001). Each layer of a layered sediment cap serves a specific function. The uppermost layer is the habitat and armoring layer. This layer is specifically designed to stabilize the cap and protect it against erosion from waves, currents, ice, prop wash, and other erosion processes. It is also designed to encourage colonization of a new benthic community. From a chemical isolation point of view, this portion of the cap is compromised as this region of the cap may be continually reworked by benthic macrofauna. Mixing from bioturbation in this layer decreases the effectiveness of this portion of the cap. The habitat zone is very important, however, as the choice of substrate in this region of the cap strongly influences the ability to restore ecological function in the sediment bed. The isolation layer provides physical and chemical isolation of contaminants in the underlying sediment to the newly colonized benthic community in the habitat layer. The increased path length for diffusion, and retardation caused by contaminant sorption to solids in the isolation layer provides decades to centuries of chemical isolation time and reduced contaminant flux. A portion of the isolation layer at the sediment/cap interface will be compromised during cap placement via mixing of contaminated sediment into the cap material during placement, or by the rapid infusion of contaminated porewater squeezed out during consolidation of the underlying sediment layer. This cap layer is termed the mixing and variation layer.

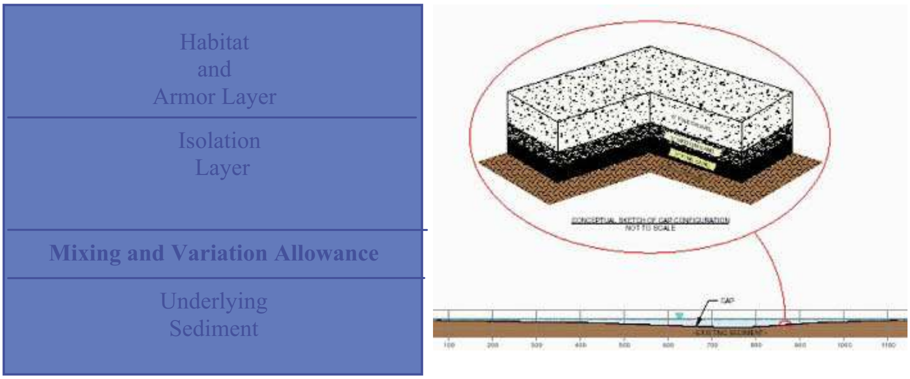


Figure 2.1. Cap layers and conceptual cap design.

2.2. ACTIVE CAP LAYERS

Materials that sequester sediment contaminants or degrade them to non-toxic products can be included in the isolation layer to improve the effectiveness of sediments caps over that provided by sand caps. Amendments designed to control groundwater seepage through a cap, sequester heavy metals or organics, or degrade organic contaminants are available. Sediment caps containing these amendments, or a suite of these amendments can significantly lower the contaminant flux from the sediments to the overlying benthic community and water column. Caps employing multiple active materials may provide the ability to address multiple contaminants. Finally, caps containing amendments capable of degrading sediment contaminants to non-toxic products can provide simultaneous isolation and in situ treatment of contaminated sediments and eliminate the negative consequences of cap failure. Some potential amendments that can be added to sand caps are presented in Table 2.1, along with the fate process encouraged by each amendment.

Table 2.1. Potential Sediment Cap amendments to Encourage Contaminant Sequestration or Degradation.

Seepage Control	**Sequestration of organics**	**Sequestration of metals**	**Degradation of organics**
Aquablok™	Activated Carbon	Apatite	Bion Soil
	Coke/Ambersorb/XAD-2	Zero valent iron	Zero valent iron
	Organo modified clay		

2.3. KEY ISSUES IN DESIGNING "ACTIVE" SEDIMENT CAPS

The ability of a sediment cap to reduce the contaminant flux from the underlying sediment into the habitat zone depends on the length of time the cap can isolate contaminants (isolation time), the overall reduction in the maximum rate of contaminant flux into the bioactive zone, and the rate of contaminant attenuation occurring in the underlying sediment. Contaminant transport through the cap occurs due to a chemical potential gradient across the cap (diffusion), and in the presence of groundwater seepage, also due to advective transport. Encouraging contaminant sorption or degradation within the cap can significantly decrease advective and diffusive transport of contaminants through the cap. Assessing the ability of a thin sorbent layer imbedded in a sand cap to mitigate contaminant transport through the cap therefore requires a quantitative description of the chemical fate and transport processes including sorption, diffusion, advection, hydrodynamic dispersion, and reaction in the underlying sediment and the cap.

There are several key issues in designing and implementing "active" sediment caps. Each of these issues is described briefly here, and further explored in later sections. Designing a sediment cap requires that the following be considered.

Defining appropriate performance criteria.

Identifying appropriate cap material to meet these criteria.

Evaluating the physicochemical and biological mechanisms affecting cap effectiveness.

Evaluating appropriate cap placement approaches.
Evaluating required armoring layer characteristics.
Developing habitat restoration criteria.

There are several ways to define the performance criteria of a sediment cap. For example, the cap can be designed to mitigate contaminant flux to some acceptable level defined by the local, state, and federal regulators. The flux may be chosen to ensure that the criterion critical concentration (CCC) for a specific sediment contaminant is never reached. Alternatively, a cap can be designed to provide a minimum isolation time (e.g. 30 years) before contaminants break through the cap layer.

Appropriate "active" cap materials must be identified for each site. The material, or combination of materials must be compatible with the contaminants present, and with the physical, chemical, and biological conditions at the site. Often, bench scale treatability studies are required to verify the sorption, degradation, and lifetime of each of the amendments being considered. The biological and physicochemical mechanisms affecting cap effectiveness must also be evaluated. For example, the rate of pore water advection due to groundwater seepage, or the effects of the sediment matrix components on the sorption strength or reactivity with sediment contaminants must be evaluated.

Once appropriate cap materials are identified, cap placement approaches must be determined. Placement techniques such as particle broadcasting, or the use of geotextiles to place "active" layers of high value material are potential placement techniques. The ability to use these methods will often be site specific. In addition to placing the cap, the required armoring layer characteristics must also be determined. The armoring characteristics will depend on site specific properties such as flow, ice, etc.

The overall goal of capping is to improve the quality of the aquatic habitat. As such, integrating sediment caps with habitat restoration is an important benefit of in situ capping. The criteria for habitat restoration must be identified and included in the design stages.

2.4. PERFORMANCE EVALUATION

For hydrophobic organic contaminants such as PCBs and PAHs, the dominant factor influencing the rate of migration through the cap is the sorption strength of the sorbent layer in the cap (retardation factor). Sorbent layers such as high organic-content soil, and thermally altered carbonaceous materials (TACMs) such as activated carbon and coke have been considered. The TACMs are known to very strongly adsorb contaminants, and PAHs and PCBs bound to these materials are less bioavailable (Talley et al. 2002, McLeod et al., 2004). Recently, the ability of a thin layer of an organic-rich soil, activated carbon, and coke at mitigating PCB transport through a sediment cap amended with each material was conducted (Murphy et al., 2005). These treatability studies determined the Freundlich parameters for sorption of 2,4,5-PCB to coke, soil, and activated carbon,

$$C_{i,solid} = K_f C_{i,water}^n \tag{1}$$

where $C_{i,\,solid}$ and $C_{i,\,water}$ are the sorbed and aqueous phase concentrations of sorbate i, K_f and n are the Freundlich parameters, and the physical properties relevant to transport through a layer of the material (effective porosity and dispersivity). The Freundlich parameter, log K_f, ranged from 4.7 for coke to 6.2 for activated carbon. The organic-rich soil (log K_f=4.9) was very similar to coke. These properties were then used in numerical models to predict the flux of 2,4,5-PCB through a 1.25-cm thick layer of each sorbent (Figure 2.2). This provides a conservative estimate for PCB flux through the cap as most PCB congeners present in sediments are higher chlorinated (more hydrophobic) PCB congeners and will have higher values of log K_f. In the absence of groundwater seepage, a 1.25-cm layer of soil, coke, and AC all provided isolation times greater than ~500 years and lowered PCB flux into the bioactive zone compared to sand. With moderate groundwater seepage (U=1cm/day), strongly sorbing high capacity sorbents such as activated carbon can still provide long isolation times (>1000 years).

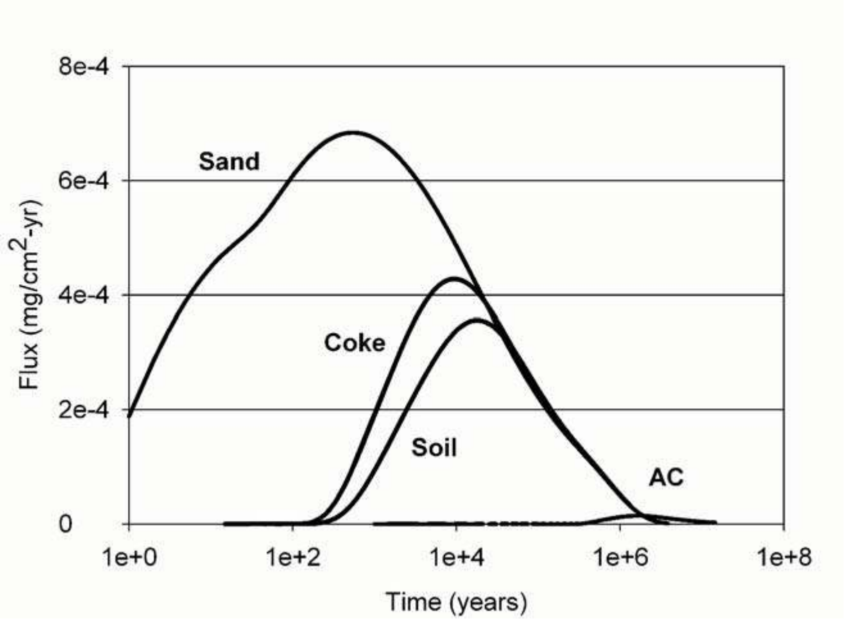


Figure 2.2a. Flux of 2,4,5-PCB for each sorbent type in the absence of advection.

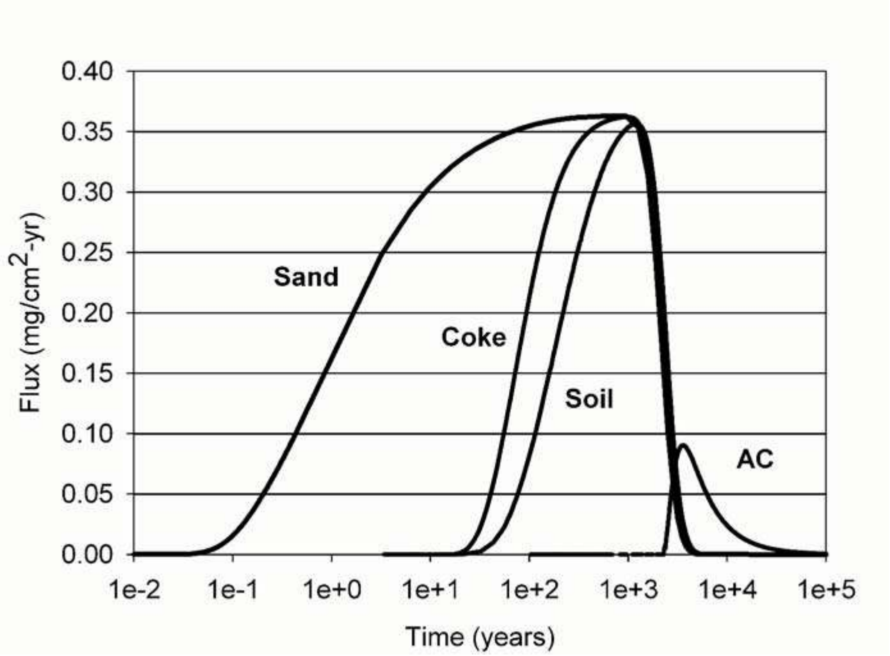


Figure 2.2b. Flux of 2,4,5-PCB for each sorbent type with groundwater advection (Darcy velocity=1cm/d).

It must be noted that these simulations employed many simplifying assumptions to assess contaminant transport (e.g. equilibrium sorption, no sorption competition), but is useful for comparing the relative effectiveness of different sorbents and providing a sense of the isolation times that may be achieved with sorbent-amended "active" sediment caps. Potentially important processes that were not addressed in this analysis include competitive sorption, sorption kinetics, and particle-facilitated transport of strongly sorbing contaminants. Competitive sorption between PCBs and dissolved organic carbon in sediment porewater may decrease K_f and lower the sorbents effectiveness. Slow sorption kinetics of PCBs onto cap sorbents may also limit effectiveness. For example, the assumption of equilibrium sorption may not be valid at high groundwater seepage velocities because the sorbent layer is thin and the residence time within the "active" layer may be too short to achieve a sorption equilibrium.

2.5. CAP PLACEMENT

In situ capping requires that a layer of clean or "active" material be placed over the contaminated sediments. Cap placement should be able to be done quickly with available construction tools (e.g. clam shell), and result in a cap of relatively uniform thickness that does not contain significant amounts of entrained contaminated sediments. The region of the cap where contaminated sediment has mixed with clean cap material will lose its effectiveness and thus must be minimized. The cap thickness and integrity must therefore be monitored during or after cap placement. The leading method to place subaqueous caps is by particle broadcasting. In particle broadcasting, a crane and clam shell is used to distribute a layer of high density granular material (e.g. sand) over contaminated sediment. Factors limiting cap placement by this methods are that thin layers (e.g. <6 inches) are difficult to place uniformly and with certainty, and that highly porous low bulk density materials (e.g. TACMs) can be difficult to place as their settling velocity is low and variable and some fraction of the material may not readily sink.

Recently, four ¼-acre test plots were placed in the Anacostia River (Wash. DC) in April 2004 to evaluate different placement and monitoring methods under realistic in-situ conditions methods, and to compare the effectiveness of traditional (sand) and innovative "active" caps relative to uncapped areas. A clam shell was used to place sand and apatite (for heavy metal sequestration) caps, and a clay Aquablock™ cap (hydraulic barrier to control tidal seepage). A 1.25 cm thick coke-filled geotextile (24 kg/m^2) accurately deployed a thin coke layer into a 15-cm subaqueous sediment cap without releasing fines or larger low density (floatable) coke particles. The sorbent layer (Figure 2.3) was placed over the contaminated sediment, followed by a 15-cm layer of clean sand to secure it and to allow a place for the benthic community to colonize. Approximately 100 m^2/hr was placed using this method, and mixing between the contaminated sediment and the cap were minimal. This is a reliable method that can be used to place thin layers of high value (e.g. nanoiron) reactive material into caps. All caps placed using the clam shell, sand, sand overlying the coke layer, apatite, and Aquablock™ were accurately placed as determined by sonar scans conducted after placement (Figure 2.4). Each material was placed uniformly and with an accuracy of ~±10cm, but with a standard deviation of less than 5 cm in all active cap layers.

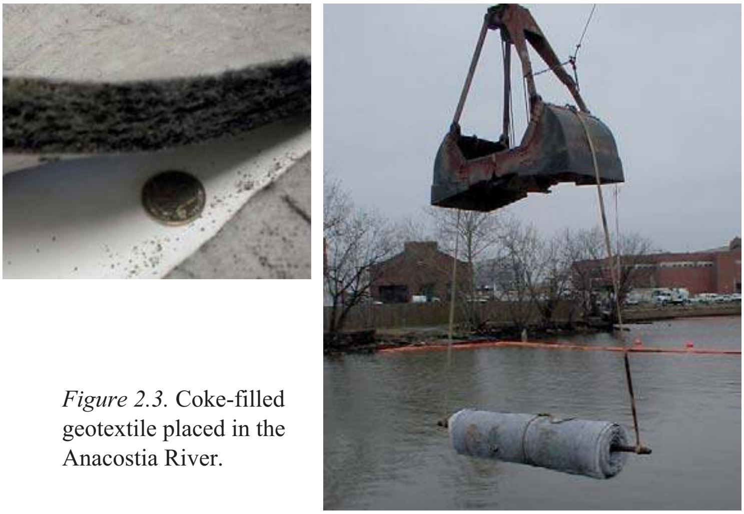


Figure 2.3. Coke-filled geotextile placed in the Anacostia River.

2.6. CAP AND TREAT SCENARIOS

Capping with traditional sand caps and with sorbent amended caps can provide very long chemical isolation times before contaminants break through the cap. A potentially highly effective approach to in situ management of sediments is to combine containment with contaminant degradation to non-toxic products. There are several ways in which cap and treat scenarios can be employed. First, the cap itself can contain "reactive"

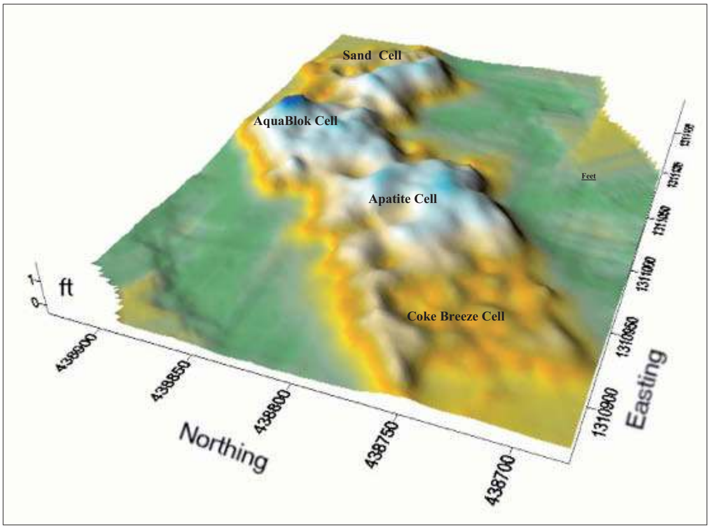


Figure 2.4. Cap heights after placement determined by sonar.

materials that degrade contaminants. This scenario is essentially a permeable reactive barrier technology similar to that used at a variety of contaminated groundwater sites. The performance issues important in groundwater sites (e.g. lifetime, production of toxic reaction intermediates, etc.) are also important in a cap application and less well understood. Second, the cap can be used to contain contaminants in the underlying sediment for long times (decades to centuries) while the contaminants in the underlying sediment are degraded through natural biological degradation, or through enhanced biological or abiotic degradation. Also, the ability to combine in situ treatment with habitat restoration is an attractive cap and treat scenario. Each of these scenarios is discussed.

2.7. IN-CAP TREATMENT

In an in-cap scenario, contaminants are degraded as they transport into the sediment cap. In theory, this type of “active” sediment cap can be used to treat the sediment porewater as it migrates through the cap and mitigates contaminant transport from the underlying sediment to the habit zone indefinitely. The concept is similar to the in situ permeable reactive barriers used in groundwater treatment (Starr and Cherry, 1994). The difficulty of this approach lies in the ability to accurately place thin layers of high value reactive material, identifying materials that afford contaminant degradation rates raid enough for use in a thin layer that may have limited reaction time, and identifying materials that remain active for extended periods of time (years to decades), or an easy method to replace the reactive material. Each of these challenges must be addressed before a robust in cap treatment method will be available.

One candidate for reactive media is zerovalent iron (ZVI). ZVI, nanoscale ZVI, or some other reducing agent can be used to encourage the reductive dechlorination of chlorinated aromatics, chlorinated solvents (Liu et al., 2005) and PCBs (Lowry and Johnson, 2004), the reduction of perchlorate (Moore, A. et al., 2003), and the reduction and immobilization of hexavalent chromium (Alowitz and Sherer, 2002). Controlled placement using a geotextile (Murphy et al, 2005) makes iron or nanoiron a viable option. Exhausted iron in a geotextile could be easily exchanged for fresh iron, especially if constructed in a funnel and gate arrangement where the active treatment area is small. A second option is to use an engineered system to provide a redox gradient from reducing to oxidizing conditions at the sediment-water interface. Composted soils or creation of a biologically active layer through the introduction of nutrients or other reactants (e.g. hydrogen) could be used to create a highly bioactive region in a cap that can biodegrade sediment contaminants as they migrate through the cap.

2.8. SUB-CAP TREATMENT

A second alternative is to use sediment caps amended with activated carbon or some other highly sorptive material, coupled with in situ treatment of sediment contaminants in the underlying sediment. The very long chemical isolation times afforded by sorbent-amended caps (Figure 2.5) allows adequate time for slow natural attenuation processes such as biodegradation that can further mitigate the risks to benthic and aquatic organisms. Engineered methods to increase the rate of biotic or abiotic attenuation (complete destruction) in underlying sediments could provide a cost-effective means to contain and treat PCB-contaminated sediments in situ. For example, contaminant attenuation rates as slow as 10^{-3} yr^{-1} ($t_{1/2}$=700 yr) in the underlying sediment can lead to significant (>90%) mass removal in the underlying sediment and decreases the maximum contaminant mass flux into the bioactive zone by a factor of 3 for an AC-amended sediment cap in the scenario evaluated in Figure 2.5). To be effective, methods to deliver and mix amendments into the contaminated sediments beneath the cap must be developed and tested. Also, methods to monitor the rate and extent of contaminant degradation beneath the cap must also be developed and tested.

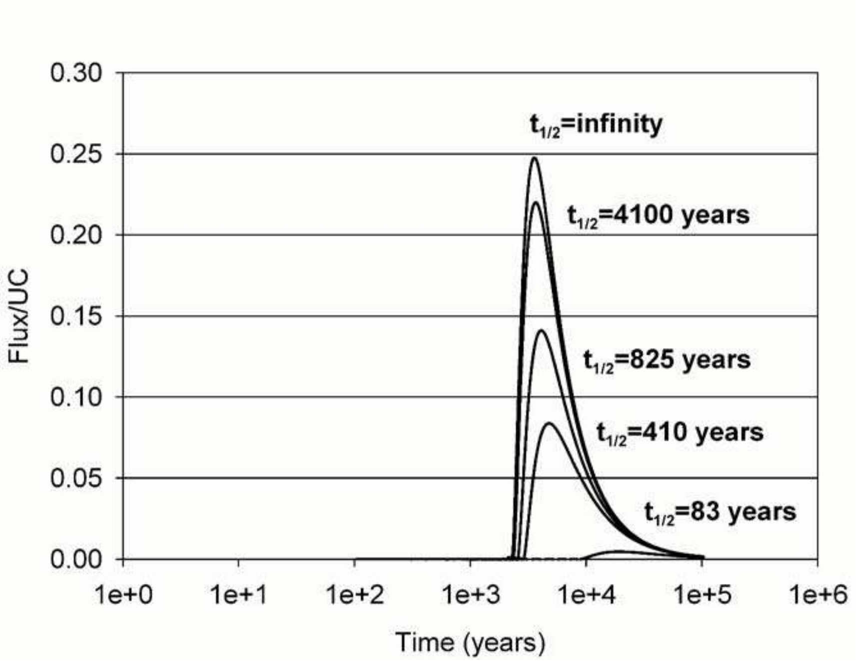


Figure 2.5. Effect of contaminant degradation in the underlying sediment on contaminant flux through the cap.

2.9. CAPPING WITH HABITAT RESTORATION

The overall goal of an sediment remediation project is to improve the aquatic habitat, and often to restore the affected habitat back to pre-contamination conditions. Cap and treat scenarios offer the potential to simultaneously manage contaminated sediments, and to restore habit. For example, a sediment cap can be designed into a site-wide habitat redevelopment designed to restore wetlands and promote fish spawning. Substrates overlying the cap layer and contaminated sediment can be selected to promote the colonization of specific species of plants, fish, or macrofauna. A conceptual model of a habitat restoration cap is shown in Figure 2.6.

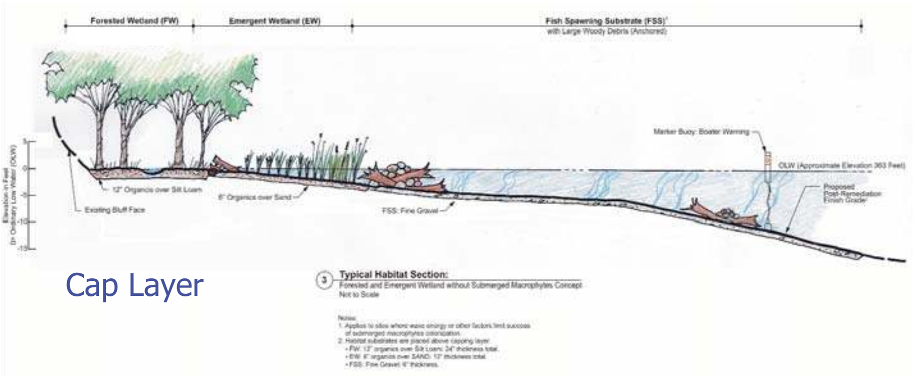


Figure 2.6. Conceptual design of sediment cap with habitat restoration.

2.10. SUMMARY AND KEY RESEARCH NEEDS

Sediment capping with "active" caps is a simple and effective in situ sediment remediation technique that can provide an alternative to dredging.

There is great potential for simultaneous containment and treatment of sediment contaminants, along with habitat restoration. Methods to accurately place thin layers of high value material within sand caps are available, which enables to potential use of high value "active" capping materials such as activated carbon and iron. Despite the great potential of "active" sediment caps and the decades of experience placing sand caps, basic research is still needed to improve cap design and performance. In particular, research is still needed to elucidate the fundamental physical, chemical and biological processes that control contaminant fate and transport and the biogeochemical gradients within a cap. Tools to measure and monitor these processes (e.g. groundwater seepage, attenuation rates) are needed. The influence of seepage of non-aqueous phase liquids (NAPL) or gas ebullition on the transport of contaminants through the cap is needed.

Continued development of cap amendments that encourage sequestration or degradation of contaminants and can last for long times under in situ conditions are also needed. A better understanding of sediment slope stability, ice scour, and other erosion processes is needed to ensure that caps maintain their integrity throughout their designed life. Finally, the integration of habitat improvement into the design of sediment caps is in its infancy and must be explored further to fully reap the benefits of this approach.

3. Phytoremediation of sediments

3.1. INTRODUCTION

Many organic and inorganic compounds including polychlorinated biphenyls, phenols, polyaromatic hydrocarbons, heavy metals are priority soil contaminants because of their toxicity and tendency to persist in soils, sediments and to escape biological degradation. The fate of contaminants in the environment is controlled by a combination of interacting processes. They can be classified as physical, chemical or biological. Physical processes are responsible for the movement of contaminants through the soil and subsurface away from their source. Chemical and biological processes determine the extent to which compounds will be transformed. In some cases the most important transformations occur abiotically, while in other cases they are mediated by microorganisms with the help of other living organisms, for example with plants. A number of plant species are able to accumulate high amounts of heavy metals in their above-ground tissues or to degrade various organic soil pollutants. Therefore phytoremediation, i.e. bioremediation using plants has received during last ten years significant attention.

However, it has become clear that phytoremediation using conventional plants has some potential for use in remediation; but also that it is encumbered by limitations that prevent widespread benefit from its significant economics. The effects of plants that accumulate metals to a level high enough to make harvesting and metal recovery economical are hampered by their mostly very small biomasses. Some toxic heavy metals are immobilised in soils and taken up only poorly by known metal-accumulating plants (Meagher 200). Studies of the use of deep-rooted trees have shown that chlorinated hydrocarbons (CHCs), such as the premier groundwater pollutant, trichloroethylene (TCE), are taken up by trees and are degraded in tree plantations. The potential of genetic engineering for enhancing the biodegradation of xenobiotic toxic compounds has been recognised since the early '80s. Initially there was hope that transgenic bacteria would provide powerful methods for remediation of environmental problems. However, this approach has failed due to two factors: inconsistent survival of genetically modified microorganisms in soil and water environments, and reluctance by regulatory authorities to approve the release of genetically modified microbes into the environment. The latter reluctance stems from the high growth rate and small size of bacteria and the ease of horizontal gene transfer among dissimilar prokaryotes. These problems can be overcome by using plants rather than microorganisms as the genetically engineered environmental cleanup biosystems. Primarily by virtue of their size, vascular plants are more easily controlled than microorganisms. By selecting sterile plants and controlling propagation by harvesting the plants prior to flowering, uncontrolled genetic releases from phytoremediation plantations can be prevented. Thus, by using plants, the promise of genetically engineered environmental cleanup systems can finally be realized (Macek et al., 2004a, b, Chaney et al., 2005, Burken et al., 2005).

During remediation of organic pollutants, it would be optimal, if the plants or plants together with a microbial consortium (Clemens et al., 2002) would be able to mineralise the compound fully to non-toxic products. It is generally accepted, that phytoremediation can be exploited on large areas, with shallow contamination accessible by the roots of species used. Decontamination of polluted water can be realized with the help of aquatic plants, different grasses are used for wetlands decontamination, contaminated sediment-derived soils usually polluted with many types of different xenobiotics can be cleaned ex-situ for example by *Salix* (Vandecasteele et al., 2005) or other plant and tree species.

Especially during the accumulation of heavy metals, but also during removal of organic contaminants the main limiting factor in large-scale exploitation of plants is the long time necessary for soil decontamination. For this reason, much effort is devoted to preparation of plants specifically tailored for phytoremediation purposes by breeding or genetic manipulations. Recently more information appeared in literature about possibilities to increase the expression of the genes already present, or introduction of bacterial or mammalian genes into plants, in order to increase the natural ability of plants to cope with xenobiotics (Krämer and Chardonnens 2001, Macek et al., 2002a, Krämer 2005). On real contaminated soil, only a few transgenic plants have been tested (Kärenlampi et al., 2000, Bizily et al., 1999, Macek et al., 2002b, Heaton et al., 1998, Pavlíková et al., 2004a).

Transgenic plant technology showed many obvious advantages over conventional plant breeding approaches for crop improvement. The discoveries allowed the engineering of new transgenic plants generating desirable products, such as enzymes, polymers and vaccines. Among new approaches, the use of transgenic plants specifically tailored for the bioremediation of organic pollutants and heavy metals have recently occurred. Beside modification of vascular plants, genetic transformation of trees (Vandecasteele et al., (2005) or monocotyledonous wetland grasses (Czako et al., 2005) was shown.

Not all plant species are equally well suited to metabolise or accumulate pollutants. Requirement for plants removing heavy metals is to grow fast in the contaminated environment, to be resistant, able to accumulate toxic metals and transfer cations or oxyanions into the harvestable (above ground) parts, or transform them into less-toxic forms (Krämer and Chardonnens 2001). At the end of the growth period, plant biomass is harvested, dried or incinerated, and the contaminant – enriched material is deposited in a special dump or added into a smelter (Kramer, 2005). The most important parameter for selection of suitable plants is not the tolerance of the plant to heavy metals, but the effectiveness in their accumulation. In addition to accumulation capacity, biomass production has to be considered in order to determine the total metal uptake. To achieve better economy of the whole process, use of energy plants or some technology crops is preferable, like hemp or flax as suggested by Griga et al. (2003). Recently also use of GM trees was described (Peňa and Séguin, 2001). Especially useful are plants able to remove more than one pollutant, because contamination, especially at industrial sites, is usually caused by a mixture of different toxic compounds. Many elemental pollutants enter to plants by basic transport systems designated for nutrient uptake. A number of xenobiotics are than stored in vacuoles as protection against their toxic effects.

Plants have developed their own systems for binding of heavy metals based largely on the synthesis of phytochelatins, described by Grill et al., (1989). Heavy metal binding in plants is normally achieved, as reviewed by Kotrba et al., (1999) by different peptides, phytochelatins and phytosiderophores. Phytoextraction of metals posses large economic opportunities because of the size and scope of environmental problems associated with metal-contaminated soils and the competitive advantages offered by a plant-based technology (Chaney et al., 1997). In case of radioactive compounds, showing high biological effect even at very low concentrations, the perfect decontamination by plants seems to be practically impossible (Soudek et al., 2003).

Complex interactions of transport and chelating activities control the rates of metal uptake and storage. In recent years, several key steps have been identified at the molecular level, enabling us to initiate transgenic approaches to engineer the transition metal content of plants (Kramer et al., 2005). Clemens et al. (2002) in excellent review summarise the determinants of metal accumulation, mobilisation, uptake, sequestration, xylem transport, discussing the role of different families of transporter proteins. Many problems are still unclear, the transport and storage forms of transition metals are largely unknown and many systems have yet to be analysed. Despite the unsolved questions, different attempts were already done to prepare transgenic plants with improved properties for phytoremediation purposes.

3.2. PREPARATION OF TRANSGENIC PLANTS

Transgenic plants used in everyday practice are nowadays represented especially by agricultural crops with increased resistance against insect pests, infections or herbicides (Koprowski and Yusibov 2001). As the next generation of genetically modified (GM) crops were introduced plants that can serve as a source of food products with increased nutritive value (e.g. with better composition and content of unsaturated fatty acids, vitamins etc.). Further, here belong plants with increased resistance against extreme weather conditions, or changes in their ability of ripening. In addition to these advantages, exploited in agricultural practice (wheat, corn, soy, rape, lemon, etc.) were prepared also GM plants able to produce pharmaceutically important products like antibodies or enzymes (McGarvey et al., 1995). Most recently as a new approach to plant protection GM plants have been prepared emiting insect sexual pheromone with the aim to disrupt mating behaviour of the target pest, thus decreasing the need for chemical pesticides (Nesnerová et al., 2004).

Genetic manipulations are thus finding increased use in plant biotechnology. Different transgenes influence the yield and qualities of harvested crops, genes regulate ripening, composition of fats and the quality of seed proteins. The studies of tissue specific promoters will allow genetic engineering to create new categories of GM plants producing the required products in a better-regulated way.

Until now most suitable vector for gene transfer into plants are the Ti plasmid of soil bacterium *Agrobacterium tumefaciens*, initiating tumours in plant tissue, and the plasmid Ri of *A. rhizogenes*, which induces hairy root formation. Hairy root cultures are exploited in phytoremediation studies for more than a decade; we used them for cadmium uptake studies (Macek et al., 1994, 1997a, b) and for PCB degradation studies (Mackova et al., 1997a, b). The exploitation of the agrobacterial gene transfer techniques is one of the main approaches for introduction of foreign genes into the plant genome (Ondrej et al., 1999), but even this already widespread technology has some inborn limitations. Agrobacterial plasmids as vectors are well suited for transformation of dicotyledonous plants, but cases of successful use on monocotyledonous plants (corn, rice, wheat) were already also described (Gelvin 1998, Czako et al. 2005). At present, the natural wild-type strains of *Agrobacterium* are no more used for the genetic manipulations, replaced by strains with modified Ti plasmids. The genes responsible for plant hormone production, that cause the formation of tumours, and genes coding opine formation, were removed, being not necessary in constructed GM plants. In the same time, genes serving as selective markers were introduced into the original T-DNA. Among the most exploited marker genes belong bacterial β-glucuronidase (GUS) and luciferase genes, and recently also the gene coding the green fluorescent protein (GFP). Because the big size of the natural Ti-plasmid caused problems in handling, the T-DNA with border sequences was introduced into smaller plasmids derived from pBR 322, which are nowadays commercially available. Within the T-DNA region of these newly prepared plasmid unique restriction sites are present, allowing introduction of constructs prepared for transfer into plant genome. Genetic construct, intended to be transferred into plants, has to contain proper promoter region (most often constitutive promoter from cauliflower mosaic virus CaMV 35S, promoter controlling ubiquinone expression in corn, or other organ or tissue specific promoters active in plants), sequence coding the required gene and termination signal. At present much effort is devoted to isolation of novel promoters inducible e.g. by some environmental effector (like the presence of heavy metals). Another technique using "natural"gene transfer is the use of viral vectors, but this is leading only to transient, not stable transformation. In cases, where the above technique is inapplicable, other methods of transfer have to be exploited. Some were developed after finding of methods allowing regeneration of a whole plant from callus culture or from a single protoplast. These techniques include direct DNA transfer (Maliga 1990), electroporation, DNA vacuum infiltration, different chemical modifications, microinjection or ballistic methods (Old and Primrose, 1994).

3.3. INCREASED ACCUMULATION OF HEAVY METALS

Among other natural mechanisms, allowing plants to grow in heavy metal contaminated environment especially many studies dealt recently with the phytochelatins. In Plant Kingdom it is a general feature to complex heavy metals by phytochelatins. These peptides are formed by phytochelatin synthase from glutathione, when plants are challenged by Cd or other heavy metals. Much effort to explanation of their formation has been devoted by Zenk and co-workers (e.g. Chasaigne et al., 2001, Oven et al., 2001).

With the aim to improve metal uptake transgenic plants were prepared with increased formation of glutathione synthase or phytochelatin synthase, what in both cases led to increased Cd accumulation (Kärenlampi et al., 2000). Transgenic plants bearing foreign genes for proteins transporting metal across membranes have been also prepared, as summarised in a review by Krämer and Chardonnens (2001). In some laboratories genes coding different types of metallothionein (mammalian, yeast, insect and human) were introduced into plants. This effort led mostly to increase of the resistance towards some heavy metals (Kärenlampi et al., 2000, Macek et al., 1996, Macek et al., 1997b), but not yet to an increase of accumulation (Liu et al., 2000). Dorlhac de Borne et al. (1998) describe cadmium partitioning in transgenic tobacco plants expressing a mammalian metallothionein gene in detail.

Reserves are not only in better understanding, possibility of modifications and improvements of normal plant mechanisms themselves, but also in other ways to improve the overall yield of the remediation process. Improved bioavailability of metals caused by changes of exudate, increased excretion of organic acids, or cooperation with rhizospheric microorganisms might be itself target of genetic engineering, or increase the performance of transgenic plants.

In order to increase the metal-binding capacity the possibility of introducing an additional metal-binding domain with high affinity towards heavy metals into the implemented protein was studied (Macek et al., 1996). In this way by combining the gene CUP1 for yeast metallothionein from *Saccharomyces cerevisiae* and the gene for the histidine anchor with high affinity to heavy metals from the commercial plasmid pTrcHis (Invitrogene) a construct has been prepared and introduced by *A. tumefaciens* into tobacco (Macek et al., 2002b). The vector was plasmid pBI121, bearing CaMV 35S promoter from cauliflower mosaic virus, the reporter gene for β-glucuronidase (GUS) and all regions necessary for successful transformation using *Agrobacterium*, purchased from CLONTECH. The tested plants involve tobacco, *Nicotiana tabacum* L., var. Wisconsin 38, as the control (WSC-38), and genetically modified lines of the same variety, bearing the transgene HisCUP (gene coding a yeast metallothionein, CUP, combined with a polyhistidine tail), CUP (gene coding a yeast metallothionein), GUS (reporter gene for ß-glucuronidase), and HisGUS (reporter gene for ß-glucuronidase, combined with a polyhistidine tail) genes under a constitutive promoter, thus enabling us to follow the heavy metal tolerance and uptake changes as function of the transgene presence. Non-transformed control – WSC-38 and four constructs of transgenic tobacco (CUP, HisCUP, GUS and HisGUS) were tested for accumulation of Cd in above ground biomass and roots. The screening test was conducted on sand nutrient media with addition of Knop's nutrient solution modified by addition of cadmium (0.2 mg $Cd.l^{-1}$ as $Cd(NO_3)_2.4H_2O$), The plants cultivated during the elongation period were harvested after 6 weeks of growth. Plant material was decomposed by dry ashing procedure and Cd concentrations in plants and roots were determined by atomic absorption spectrometry using Varian SpectrAA-300 (Pavlikova et al., 2004a, b).

The model pot experiment was set up using two soils with different Cd content – unpolluted Chernozem and Cambisol with contamination caused by the atmospheric emissions. In experiment, 5 kg of soil were thoroughly mixed with 2 g N applied as NH_4NO_3. P and K were added as K_2HPO_4 at a rate of 0.44 g P and 1.1 g K per pot at the same time. The pots were irrigated by deionised water and humidity of the soil was kept on 60% of maximal water capacity for duration of the whole experiment. The plants were cultivated for three months. To evaluate statistical significance of the changes during experiment, each treatment was carried out in four replicates analysed separately.

3.3.1. *Screening test in sand medium*

Presence of introduced genes in plant tissue was proved by PCR, RT-PCR respectively (Macek et al., 2002b). The abilities of transgenic plants were tested first in sand medium containing defined amount of cadmium. The results showed that while control WSC-38 whole plants accumulated in average 15.8 mg $Cd.kg^{-1}$ in leaves and stems and 19.9 mg $Cd.kg^{-1}$ in roots (Pavlikova et al., 2004a) the presence of the CUP1 yeast metallothionein gene did not increase the accumulation much above the average of control. Cd concentration in HisGUS constructs did not differ from control treatment. The HisCUP construct proved to have positive effect on Cd accumulation. The Cd content in above ground biomass was increased by 90% comparing to the non-transformed control and the Cd content in roots was decreased by 49%. Distribution of this element among leaves, stems and roots showed majority of Cd in leaves of both treatments (87% from total uptake by HisCUP and 71% by control). Uptake by stems was the lowest – 5% control, 3% HisCUP. Uptake by roots also showed the difference between control and HisCUP construct (control 24%, HisCUP 10% from total uptake).

Table 3.1. Cd content in tobacco plants growing in sand medium (Pavlíková et al., 2004a).

Cd dose (mg Cd.l^{-1})	Cd content (mg . kg^{-1})			
	WSC 38		HIS-CUP	
	Roots	Above-ground biomass	Roots	Above-ground biomass
0.2	13.6 ± 2.9	16.6 ± 5.9	11.6 ± 6.2	30.1 ± 3.5
0.6	34.4 ± 4.17	46.8 ± 4.6	36.8 ± 2.9	74.7 ± 3.3
1.8	142.5 ± 0.7	125.5 ± 2.1	150.0 ± 6.4	144.6 ± 1.3
5.4	520.0 ± 15.5	259.0 ± 4.5	514.0 ± 23.6	336.0 ± 5.9
16.2	1908.5 ± 51.2	647.5 ± 111.6	1886.8 ± 100.5	736.5 ± 269.2

The Cd content in above ground biomass of tobacco growing at Cd concentration of 0.2 mg Cd.l^{-1} was increased by 80 % compared to the non-transformed control (Table 3.1). This result confirmed our previous experiments that accumulation of cadmium significantly increased in plants bearing the transgene coding the polyhistidine cluster, combined with yeast metallothionein (Macek et al., 2002b). Significantly increasing accumulation was also determined in HisCUP tobacco growing in Cd concentration of 0.6 mg Cd.l-1. Cadmium content in roots of control tobacco did not differ from transgenic plants and it was lower compared to the above ground biomass. Next three doses 1.8, 5.4 and 16.2 mg Cd.l-1 caused lower differences of Cd contents in plant above ground biomass and roots between control and HisCUP plants. Dose of 5.4 mg Cd.l-1 induced cadmium toxicity symptoms in leaves of control tobacco that resembled Fe chlorosis. The highest dose of Cd (16.2 mg Cd.l-1) in sand medium depressed root and plant growth and was lethal for this control treatment. HisCUP tobacco showed increasing plant resistance against stress response induced by cadmium. Both doses were not toxic for transgenic plants.

3.3.2. *Model pot experiment in real contaminated soil*

Experiments in contaminated soil gave better picture about properties of transgenic plants and showed their real capacity to accumulate heavy metal and survive in its presence, both affected primarily by bioavailability of Cd. This is different in sand medium and in real contaminated soil.

The bioavailability of Cd to plants can be more accurately predicted determining available portion of Cd than the soil total content (Adriano, 2001). Before the experiment plant-available Cd formed 8% from total Cd content in polluted Cambisol. Our results did not show changes of available Cd content after harvest of tobacco plants in this soil (Pavlikova et al., 2004b). Unpolluted Chernozem contained 0.56% available Cd from total Cd content. Significant decrease of Cd was observed only in this soil after harvest of transgenic tobacco.

Both yield and content of dry matter of transgenic plants were not significantly differing compared to control. The most important factor affecting Cd content in tobacco plants cultivated in this experiment was the original Cd content in soils. Content of Cd in tobacco growing on polluted Cambisol with high portion of available Cd was 14-17 fold higher compared to Chernozem. The content of Cd in the above ground biomass of transgenic tobacco cultivated in non-polluted soil increased by 75% compared to control plants and by 45% in polluted soil (Table 3.2). Very important fact for phytoremediation is, that most of the accumulated cadmium was stored in the leaves, while the metal content in roots was lower comparing to the control plants. Previously it was shown by King and Hajjar (1990) that Cd concentrations in tobacco declined markedly with higher leaf position on the stalk. This general decline with increasing leaf height in the tobacco plant suggests that Cd has limited mobility within the plant and its concentration is related to the age of the leaf.

Table 3.2. Cd content in tobacco plants growing in pot experiment (Pavlikova et al., 2004b).

	Cd content (mg . kg^{-1})		
Soil	Treatment	Roots	Above ground biomass
Chernozem	WSC 38	0.24 ± 0.12	1.25 ± 0.60
	His CUP	0.13 ± 0.03	2.19 ± 0.95
Cambisol	WSC 38	3.61 ± 0.65	21.33 ± 4.84
	His CUP	2.50 ± 0.41	31.43 ± 5.65

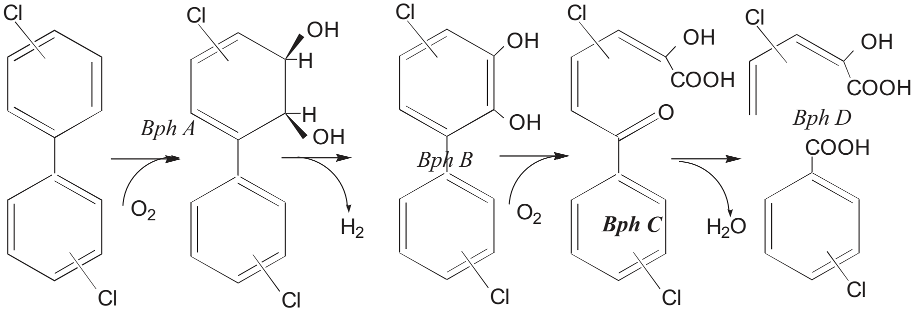
Uptake of heavy metals by plants is characterized by transfer factors values (Kiekens and Camerlyck, 1982). Transfer factor is calculated as quotient of metal total content in plant biomass and in soil. According to Kloke et al. (1984) Cd transfer factor is 1-10. Higher values are desribed for leafy plants. Tlustos et al. (1998) calculated transfer factor values 1.50 - 3.59 for spinach leaves and 1.65 – 3.32 for spinach roots. Our results showed high transfer factor for tobacco, mainly for above ground biomass of transgenic plants 5.26 – 6.65 (Pavlikova et al., 2004a).

The comparison of genetically modified lines of tobacco with control plants showed an increase of resistance and good growth capability in cadmium contaminated soil, together with increased accumulation. Already before transformation of plants prepared constructs were tested in *E. coli* in a series of growth experiments, and together with improved resistance a substantial increase of Cd accumulation was found (Macek et al., 1997b, 1998) already in bacterial biomass.

3.4. GM PLANTS FOR REMOVAL OF PCB

Polychlorinated biphenyls represent another important xenobiotic contaminating environment in many, especially in industrialised countries. Even now, 35 years after their production was banned in civilised countries, and 18 years after finished in former Czechoslovakia, these persistent compounds still represent a danger for biological diversity and for man. Being taken up by plants, PCBs can enter food chain and accumulate mostly in fat tissues. The ability of plants to metabolise them has been already proved, and is further studied (Kucerova et al., 2000). In our laboratories, we have a quite complex approach to PCB bioremediation (Demnerova et al., 2002, Mackova et al., 2001), which includes studies on enzymes involved (Chroma et al., 2003), products formed and attempts to introduce bacterial genes from the bacterial bph operon (Francova et al., 2003, Sura et al., 2005)). Starting with screening of different plant species *in vitro* (Mackova et al., 1997b), identification of plants growing on long-term contaminated soil (Ryslava et al., 2003), and characterisation of cooperation between degrading bacteria and plants, the best suited models were selected. Some of the products formed in the first, activation step of the detoxification pathway in plants were already identified as hydroxychlorobiphenyls (Kucerova et al., 2000). We proved that several derivatives of these hydroxylated monochlorobiphenyls can serve as substrates for bacterial enzymes of the bacterial biphenyl operon, and prepared plants bearing *gene C* from the bacterial biphenyl operon, responsible for opening of the ring of recalcitrant PCB molecules (Francova et al., 2004) (Fig. 3.1), could degrade them. Attempts to genetically improve the PCB-converting ability of plants are a further example of this approach in environmental remediation. While plants detoxify PCBs by hydroxylation and conjugation to hydrophilic molecules, followed by storage, some bacteria can open the biphenyl ring, thus allowing further steps leading to mineralization (see Fig 3.1, step 3). For this reason, as described in Borovka et al. (1998) and in Macek and Macková (1999), a bacterial gene (*bph C*) responsible for biphenyl ring opening (see Fig. 3.1) was chosen for these attempts.

VI



I II III IV

V

Figure 3.1. Bacterial degradation of PCB – upper pathway (Hein et al., 1998, Pieper 2005).

I - biphenyl, II - 2,3-dihydroxy-4-phenyl-hexa-4,6-diene, formation catalyzed by gene *bphA*, III - 2,3-dihydroxybiphenyl , formation catalyzed by enzyme coded by gene bphB, IV - 2-hydroxy-6-oxo-6-phenyl-hexa-2,4-dienoic acid, formation catalyzed by enzyme of gene bphC, V - benzoic acid, VI - 2-hydroxy-penta-2,4-dienoic acid, formation catalyzed by enzyme coded by gene bphB.

This gene from *Comamonas testosteroni* B356 is coding the enzyme responsible for PCB ring opening, 2,3-dihydroxybiphenyl-1,2-dioxygenase, characterised by Sylvestre and co-workers (Hein et al., 1998). Cloning into *Agrobacterium tumefaciens* and plant transformation was performed by standard methods (Francova et al., 2003). Constructs harbored the constitutive promoter CaMV 35S.

For detection and other physiological studies constructs coding fusion proteins *bphC* and reporter genes for green fluorescent protein (GFP), luciferase (LUC) or glucuronidase (GUS) and His tail (6x histidines) were also designed and prepared (Sura et al., 2005).

The transgenic plants were selected after transferring the regenerants to selective medium. The best yield of regenerated transformed plantlets was obtained on medium according to Linsmeier and Skoog (LS medium) containing kanamycine or hygromycine (Sura et al., 2005) and supplemented by naphtaleneacetic acid and kinetin, as suggested by Macek et al. (1998).

3.4.1. *Testing of the properties of GM plants containing bphC gene*

Successful transformation of plant tissue was confirmed on different levels of gene expression:

A) First successfully transformed plantlets proved kanamycine or hygromycine resistance and regenerants grew on LS medium in presence of antibiotic (kanamycine or hygromycine). In particular also by root formation of selected regenerants on rooting-supporting LS medium in presence of kanamycine (hygromycine).

B) By isolation of plant DNA and RNA, identification of *bphC* fragment after PCR or RT-PCR amplification with specific primers (Sura et al., 2005) and electrophoresis in agarose gel and comparison of its size with bacterial gene (Fig. 3.2, 3.3),

C) Histochemical detection of reporter genes cloned in fusion with *bphC* (glucuronidase and luciferase) – see Fig 3.5.

D) Expressed fusion protein BphC/GFP was detected by Western blot analysis / see Fig. 3.4.

E) Growth and better resistance to high PCB concentration (see Fig. 3.6).

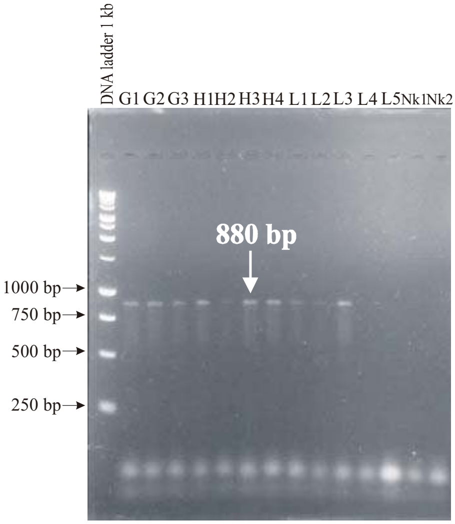


Figure 3.2. Agarose-gel electrophoresis after PCR of plant DNA transformed with bacterial *bphC* gene.

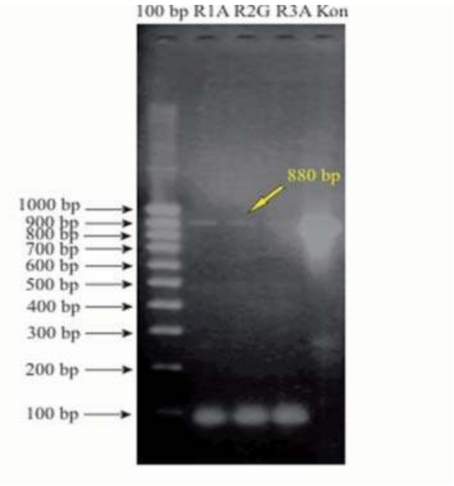


Figure 3.3. Agarose-gel electrophoresis after PCR of cDNA obtained after RT-PCR of plant RNA containing *bph C* gene. The plant RNA was isolated, treated by DNase I, and further RT-PCR proved presence of appropriate RNA (see Figure 3.3).

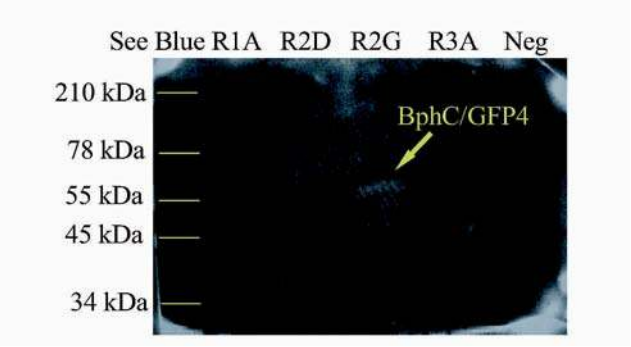


Figure 3.4. Western blot with commercial antibody against GFP. Protein extracts were prepared from different clones (R1A, R2D, R2G, R3A) grown after transformation with *Agrobacterium tumefaciens* bearing fused genes bphC/GFP and negative control from nontransformed tissue.

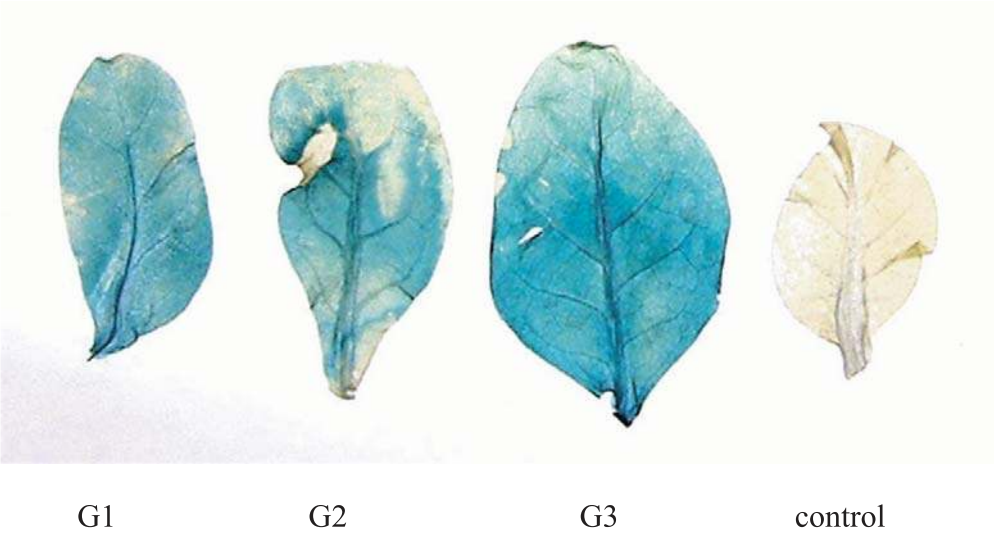


Figure 3.5. Histochemical detection of glucuronidase in transgenic plants (G1, G2, G3) and nontransformed plant used as a negative control.

Figure 3.6. Growth of transgenic (3KFC) and nontransgenic (WSC) tobacco on agar medium with 250 ppm PCB. The figure shows the growth of nontransgenic (WSC) and transgenic (3KFC) plants (vegetatively multiplied) on medium containing 250 ppm of PCB. It can be seen that transgenic culture exhibits much higher resistance to high PCB concentration than nontransgenic ones.

Transgenic plants originally grown on agar medium were transferred to normal soil and grew to adult plants during three months period. Grown seeds were harvested and then tested for the ability to germinate on medium with kanamycine and PCB to select those plants, which are highly resistant to PCB (Macek et al., 2005).

Finally it can be concluded that engineered organisms used in this study were previously proven to be stable and to survive in contaminated media. At present, experiments are underway to evaluate the performance of GM plants on media with PCBs added, and to follow the formed products.

3.5. CONCLUSIONS

Not all waste types and site conditions are comparable. Each site must be individually investigated and tested. Engineering and scientific judgment must be used to determine if a technology is appropriate for a site, as EPA repeatedly states (Anonymous 1998). Engineered degradation by transgenic plants proved to be a possible remediation technology that leads to more favorable contaminant fate and reduced toxicological concerns.

The most important parameter for selection of suitable plants is not the tolerance of the plant to the contaminants, but the effectiveness in their accumulation or degradation. In addition to accumulation capacity, biomass production must be considered in order to determine the total metal uptake. We tried to address these problems by preparing GM plants with improved properties. Our results allow for moderate optimism, the GM tobaccos with *bphC* express the bacterial PCB cleavage gene, and the HisCUP plants exhibit better Cd accumulation, higher resistance and better transport into harvestable parts. Instead of tobacco as a useful laboratory model, other species can be considered to be tailored according to requirements of specific contaminated sites (Griga et al., 2003), especially technological crops are interesting candidates for this task.

Only the decreasing of the amount of environmental contamination can prevent the toxic compounds entering the food chain, help to conserve the biological diversity (which is surely more endangered by the contaminated environment than by the use of genetically modified plants) (Macek et al., 2002a), and this way contribute to the sustainable development. Other advantages of phytoremediation systems are still germane to transgenic modifications, including: the cost effectiveness for large areas and the natural aesthetic appeal (Burken et al., 2005). Our experiments illustrate two viable approaches to prepare plants with properties suitable for exploitation in biological remediation of soils contaminated by heavy metals or polychlorinated biphenyls.

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BIOLOGICAL ASSESSMENT AND REMEDIATION OF CONTAMINATED SEDIMENTS

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Abstract- Various approaches to clean contaminated aquatic environments have been proposed. In recent years, natural attenuation has received increasing attention and it is generally accepted that microorganisms are the principal mediators of the natural attenuation of many pollutants. However, the complexity of environmental systems such as sediments requires a multifaceted approach to understand microbial processes and their potential. This is even more so under *in situ* conditions, where the activity of pollutant degrading microorganisms is generally slow, partial and

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constrained spatially and/or temporally. Recent developments in molecular biology and genomics are offering tools to explore microbial processes at a level that encompasses the genetic characteristics of the local microbial players, culturable or not, as well as their organization into complex communities and their interactions both with each other and with the target chemicals. It is now possible to study microbes directly in their environments at the population level as well as at the single cell level and to link biology to geochemistry. Integrative knowledge from culture independent studies based on functional characters and assessment of the diversity and quantity of catabolic genes in response to pollution, will allow a deeper understanding of and a rational intervention in environmental processes. Moreover, the use of genomic libraries to retrieve genes from natural bacterial communities without cultivation will allow a breakthrough in accessing new microbial capabilities. In this chapter, the main features, advantages and limitations of these innovative approaches to the biomonitoring and analysis of intrinsic bioremediation potential of polluted environments and sediments are critically reviewed. Then, the potential of the same strategies in the integrated chemical, physical and biological monitoring and characterization of polluted sediments subjected to natural decontamination is shown through the description of the main results of case studies performed on a) polychlorinated biphenyl (PCB)-contaminated marine sediments of the Porto Marghera area of Venice Lagoon (Italy) in which the occurrence of PCB-reductive dechlorination processes has been demonstrated for the first time in the literature, b) sediments contaminated by chlorinated aliphatic hydrocarbons (CAHs) collected from different positions of the eutrophic river Zenne (Vilvoorde, Belgium), where they have been found to act as a natural biobarrier for the CAHs occurring in the groundwater that is passing through the sediment zone, hereby reducing the risk of surface water contamination, and c) other environmental contaminated systems subjected to ex-situ and in situ active bioremediation, where these processes are described on the basis of the experience accumulated in pilot and real-life systems.

Keywords: polychlorinated biphenyls (PCBs), chlorinated aliphatic hydrocarbons, bioaugmentation, biostimulation, reductive dechlorination, biosensors, PCR, DGGE, T-RFLP, metagenomics, sulfate-reducing bacteria, fingerprinting methods, *exsitu* treatment, *in situ* treatment, engineered bioremediation systems.

1. The detection and characterization of microbial processes in sediments

Microbes critically impact and mediate nearly every major biogeochemical cycle on Earth. Microbial interactions that occur on the scale of microns can regulate global processes on planet-wide scales. As an example, the decomposition and transformation of both organic and inorganic compounds in sediments is almost completely carried out by microbes. The crucial role of microbes in the environment had been underestimated since the first discovery of these microscopic living forms. The main reason for this was the difficulty to access the astonishing diversity of microbes since the classical approaches based on their cultivation in the laboratory are biased and inadequate. There is a wide consensus among microbiologists that more than 90% of microbes are refractory to the classical approaches of cultivation[1]. Recent advances in understanding the diversity and importance of microbes, using culture-independent approaches, has led without any ambiguity to the conclusions that microbes are by far the most dominant living forms in terms of number of species, number of individual cells, total biomass, diversity of habitats, and diversity of cellular chemistries. Thus the environmental impact of microbes is being taken seriously and stands in the forefront of studies aiming at understanding the global environmental changes. Microbes dominate animal life in sediments and aquifers, and they are responsible for major fluxes of organic and inorganic nutrients. They are involved in processes as different as anaerobic methane oxidation[2], uranium accumulation[3], pesticide degradation[4], and polychlorinated biphenyl degradation[5].

1.1. HOW TO MASTER INCREASING COMPLEXITY

The complexity of microbial life in both natural and man-impacted habitats requires a multidisciplinary approach. In addition to their large diversity, microbes live in interaction with the different components of their environments. Figure 1.1 shows schematically the inherent complexity of a polluted sediment undergoing bioremediation. Several factors affect the process of biodegradation (bioavailability, physico-chemical conditions, composition of microbial communities, etc.). The characterization of such a complex biotope is now feasible using tools offered by such disciplines as ecology, biogeochemistry, physiology, biochemistry, genetics, process engineering, molecular biology, and metagenomics. However the relevance

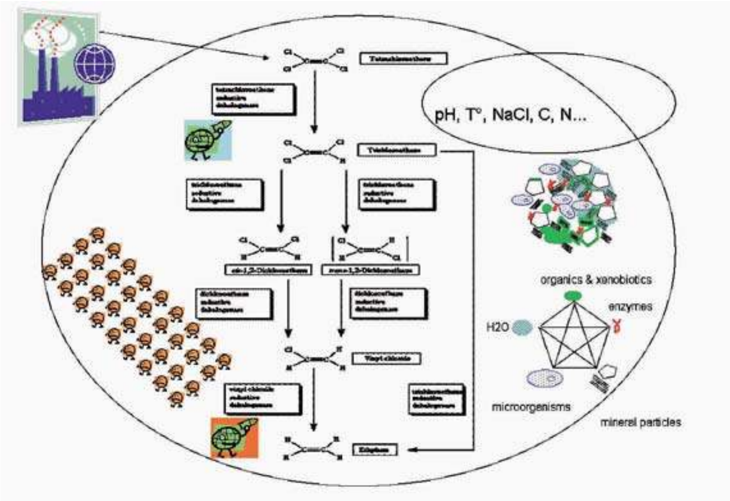

Figure 1.1. Schematic representation of the complexity of polluted sediments undergoing bioremediation. The anaerobic dechlorination of tetrachloroethene (PCE) is used as an example of productive microbial metabolism leading to site cleanup.

and efficiency of each discipline depends on the scale of study (pure culture, laboratory bioreactors, or field studies). Figure 1.2 shows the relevance of each discipline to the field studies (adapted from E.L.Madsen[6]). The most striking discipline that has recently emerged is metagenomics, defined as the culture-independent genomic analysis of entire microbial communities. Metagenomics provides access to the pool of genomes of a given environment using a comprehensive survey of nucleotide sequence, structure, regulation, and function. Furthermore, direct genomic cloning offers the possibility to retrieve unknown sequences or functions, whereas methods relying on PCR amplification require prior knowledge of the sequence of genes for the design of primers[7,8].

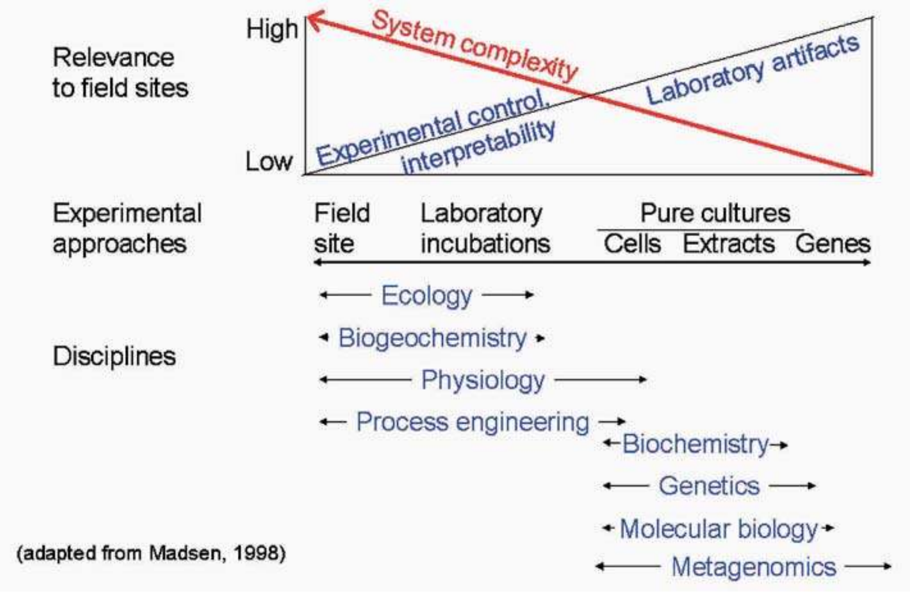

Figure 1.2. Disciplines involved in understanding microbial processes in sediments and their relevance to different scales of studies.

1.2. EXPERIMENTAL APPROACHES

Studying microbes in sediments involves several approaches that can be summarised by the following flowchart.

Phase 1: Environmental sampling: physiological studies (adapted from E. L. Madsen[9])

1. Sediment, soil, water or sludge in field site.
2. Aseptically remove, contain, transport to laboratory.
3. Divide into replicate live and abiotic treatments.
4. If appropriate, add radiolabelled or unlabelled organic compound of interest.
5. Use analytical chemistry or physiology tools to periodically measure consumption or production.
6. Compare time courses of live and abiotic treatments.
7. Interpret and consider genetic studies in Phases 2 and 3.

Phase 2: Specialized microbiological studies using culture-based methods

1. Isolate pure culture expressing activity found in Phase 1.
2. Characterize growth, cell yield, sequential induction, and other characteristics during pollutant metabolism.

3. Extract and identify metabolites, enzymes and cofactors linked to pollutant metabolism.

4. Study metabolites, enzymes and cofactors in cell-free system.

5. Determine portion of genomic or plasmid DNA coding for pollutant metabolism by screening a cloned DNA library, by transposon mutagenesis or by other procedure.

6. Do hybridization, restriction mapping and sequencing DNA analyses seeking ORFs, phylogenetic relationships to similar genes, etc.

7. Elucidate details of gene expression and regulation via various genetic & molecular techniques (transposon mutagenesis, expression clones, insertional inactivation, inducer/reporter experiments).

Phase 3: Specialised microbiological studies using culture-independent approaches

1. Using a few grams of sediments, extract the whole genomic material (DNA and/or RNA).
2. Clean up the nucleic acids.
3. Proceed through PCR or in situ hybridization to study species or consortia of interest.
4. Use fingerprinting methods (DGGE, TRFLP, etc.) and clone libraries to study microbial diversity.
5. For genomic and metagenomic studies shear the nucleic acid fragments and clone them in vectors such as fosmids and BACs.
6. Use the function-driven approach or the sequence-driven approach to explore the phylogenetic and physiological diversity of the sediment biotope.

1.3. MOLECULAR TOOLS TO DETECT AND MONITOR MICROBES

The development of molecular tools has allowed more precise and sensitive techniques to be applied for microbial detection in sediments compared to classical microbiological methods. The most popular and widely used molecular techniques are those based on polymerase chain reaction (PCR). PCR techniques targeting specific portions of genes have been used to detect microbes and to explore microbial diversity in sediments, aquifers and soil samples. Several studies have shown the advantage of using PCR to detect and quantify the presence of microbes. As an example El Fantroussi et al[10] have used 16S rRNA-based techniques to study the biodegradation of chloroaromatic compounds in a simulated aquifer. The utilisation of PCR in parallel with biodegradation in microcosms showed an unambiguous role of bioaugmentation as a strategy for the bioremediation of polluted sediments under constraints of fluid flow patterns, depth of matrix and presence of competing indigenous microbial communities.

Hendrickson et al.[11] examined the environmental distribution of organisms belonging to the *Dehalococcoides* group, known to be major players in the anaerobic transformation of chlorinated compounds, and their association with chloroethene-contaminated sites. Samples from 24 chloroethene-dechlorinating sites scattered throughout North America and Europe were tested for the presence of members of the *Dehalococcoides* group by using a PCR assay developed to detect *Dehalococcoides* 16S rRNA gene (rDNA) sequences. Sequences identified by homology to the *Dehalococcoides* group were detected at 21 sites. Full dechlorination of chloroethenes to ethene occurred at these sites. *Dehalococcoides* sequences were not detected in samples from three sites at which partial dechlorination of chloroethenes occurred, where dechlorination appeared to stop at 1,2-*cis*-dichloroethene. Phylogenetic analysis of the 16S rDNA amplicons confirmed that *Dehalococcoides* sequences formed a unique 16S rDNA group[11].

Other studies have demonstrated the power of combining molecular techniques with classical approaches to understand microbial processes in sediments. De Lipthay et al.[12] evaluated how the in situ exposure of a subsurface aquifer to phenoxy acid herbicides at low concentrations (<40 $\mu g\ l^{-1}$) changes the local microbial community composition. Sediment and groundwater samples collected from a herbicide-exposed area and unexposed controls were analyzed for the presence of general microbial populations, *Pseudomonas* bacteria, and specific phenoxy acid degraders. Both culture-dependent and culture-independent methods were applied. The abundance of microbial phenoxy acid degraders (10^0 to 10^4 per gram of sediment) was determined by most probable number (MPN) assays, and their presence was only detected in herbicide-exposed sediments. Similarly, PCR analysis showed that the 2,4-dichlorophenoxyacetic acid degradation pathway genes *tfdA* and *tfdB* were only detected in sediments from contaminated areas of the aquifer. Different populations of *tfd* genes have been found, suggesting that the in situ herbicide degradation was caused by the activity of a heterogeneous population of phenoxy acid degraders. The number of *Pseudomonas* bacteria was higher in sediments subjected to high levels of phenoxy acid. Furthermore, high numbers of CFU compared to direct counting of 4',6-diamidino-2-phenylindole-stained cells under the microscope suggested an increased culturability of the indigenous microbial communities from acclimated sediments. The findings of this study demonstrate that continuous exposure to low herbicide concentrations can markedly change the bacterial community composition of a subsurface aquifer.

Anaerobic methane oxidation is a process that effectively controls emission of methane from many anaerobic environments into the atmosphere and thus plays an important role in the global methane budget[2]. Microbially mediated oxidation of methane in anoxic marine systems is a globally significant process, with up to 90% of the oceanic methane production recycled in anaerobic marine sediments[13]**.** Other studies have shown that microorganisms living in anoxic marine sediments consume more than 80% of the methane produced in the world's oceans. In addition to single-species aggregates, consortia of metabolically interdependent bacteria and archaea were found in methane-rich sediments. A combination of fluorescence in situ hybridization and secondary ion mass spectrometry showed that cells belonging to one specific archaeal group associated with the *Methanosarcinale*s were all highly depleted in C-13. This depletion indicates assimilation of isotopically light methane into specific archaeal cells[2]. Anaerobic methane oxidation was also investigated in marine sediment from Aarhus Bay, Denmark[14]. Measured concentration profiles for methane and sulfate, as well as in situ rates determined with isotope tracers, indicated that there was a narrow zone of anaerobic methane oxidation about 150 cm below the sediment surface. Methane could account for 52% of the electron donor requirement for the peak sulfate reduction rate detected in the sulfate-methane transition zone. Molecular signatures of organisms present in the transition zone were detected by using selective PCR primers for sulfate-reducing bacteria and for *Archaea*. One primer pair amplified the dissimilatory sulfite reductase (DSR) gene of sulfate-reducing bacteria, whereas another primer (ANME) was designed to amplify archaeal sequences found in another study of sediments from the Eel River Basin, as these bacteria have been suggested to be anaerobic methane oxidizers[15]. This study is a nice illustration of combining chemistry to molecular biology to bring into stark evidence globally significant biogeochemical processes that are mediated by diverse coexisting and interacting microbial populations[14].

1.4. SYNTROPHY

Anaerobic oxidation of methane, also called reverse methanogenesis, in sediments illustrates once again the importance of syntrophy in the microbial world in general and in anaerobic processes in particular. As in the process of methane production, methane oxidation occurs in ecological niches where archaea, responsible for methane oxidation; live in association with sulfate reducers. Syntrophy was first discovered in 1967 as a synergistic relationship between an ethanol-oxidizing bacterium and a methanogen. In the late 1970s, the importance of syntrophic relationships became established through culture-based studies of methanogens interacting with bacteria that oxidized lactate, butyrate, or propionate. Biochemically, these bacteria dispose of reducing equivalents by reducing protons or bicarbonate to H_2 and formate, respectively. Both products serve as electron carriers in interspecies H_2 transfer and interspecies formate transfer between syntrophic acetate-producing bacteria and methanogens. Since their original discovery, many other syntrophic systems have been identified, and the central role of syntrophic interactions in environmental samples and engineered anaerobic bioreactors is now widely recognized. Syntrophy (or syntrophism) can be defined as the interaction of two or more populations that satisfy each other's nutritional needs[16]. This strategy of "feeding together" is involved in the anaerobic breakdown of various sugars, amino acids, and aromatic compounds. El Fantroussi et al.[10] used *Desulfomonile tiedjei* for bioaugmentation of a simulated aquifer polluted with 3-chlorobenzoate. This dechlorinating bacterium was isolated from a syntrophic association with a benzoate oxidizer and a H2-consuming methanogen (Figure 1.3).

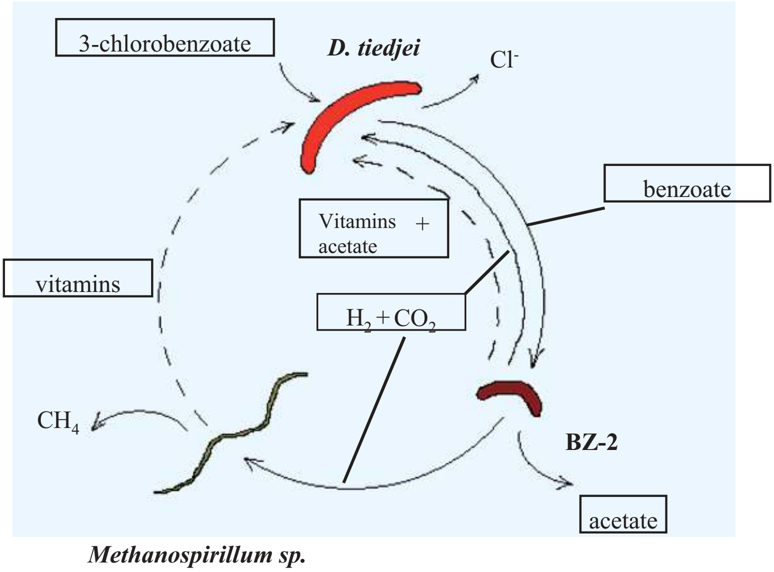

Figure 1.3. Example of a bacterial syntrophic association.

D. tiedjei is a scavenger which relies on electron donors and vitamins from other organisms and uses a broad range of electron acceptors including 3-chlorobenzote[17]. The establishment of this strain in the simulated aquifer was evaluated with physiological microcosms and PCR detection of its 16S rRNA signatures. There was a close relationship between 3-chlorobenzoate dechlorination, methane production and PCR detection[10].

1.5. ENVIRONMENTAL MONITORING – BIOSENSORS

To fully understand microbial processes in sediments there is a need for new, fast, specific, accurate, precise and cost effective methods for field assays to monitor both chemical species and ‘effect related’ processes (e.g. xenoestrogens). Several techniques have been developed for environmental monitoring of chemical species. These methods include ultra-sensitive techniques based on immunoassays as well as whole-cell (mainly by using genetically engineered microorganisms “GEM”) biosensors to assess toxicity / bioavailability that can be applied to both metals and organics. However there are still some technical barriers related to sample treatment and cleanup prior to instrumental analysis. These procedures are also time-consuming and often not suited for *in situ* field monitoring, e.g. sediments. To achieve this goal Acha et al.[18] have developed a novel attenuated total reflection-Fourier transform infrared (ATR-FTIR) sensor and applied it to the continuous on-line monitoring of a dechlorination process. This optical sensor was developed to measure noninvasively part-per-million (ppm) concentrations of trichloroethylene (TCE), tetrachloroethylene (PCE), and carbon tetrachloride (CT) in the aqueous effluent of a fixed-bed dechlorinating bioreactor, without any prior sample preparation (Figure 1.4).

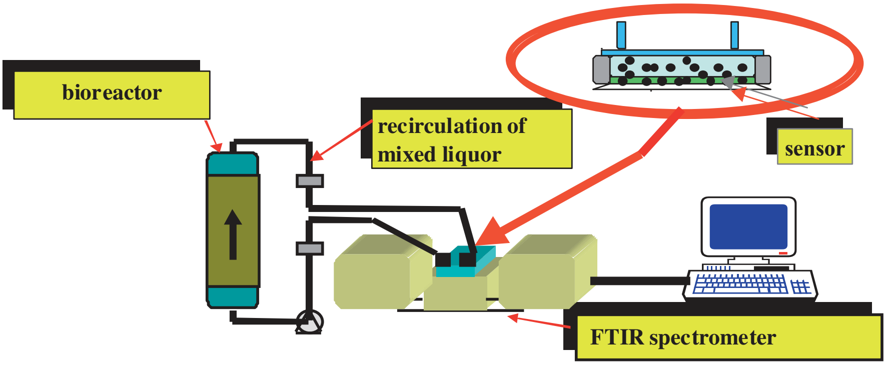

Figure 1.4. ATR-FTIR sensor (shown inside the circle) coupled to an anaerobic dechlorinating bioreactor for continuous on-line monitoring of chlorinated organic compounds.

The sensor was based on an ATR internal reflection element (IRE) coated with an extracting hydrophobic polymer, which prevented water molecules from interacting with the infrared (IR) radiation. The selective diffusion of chlorinated compound molecules from aqueous solution into the polymer made possible their detection by the IR beam. With the exclusion of water the detection limits were lowered, and measurements down to 2, 3, and 2.5 mg/L (ppm) for TCE, PCE, and CT, respectively, became possible. Before coupling the ATR-FTIR sensor to the dechlorinating bioreactor, preliminary spectra of the chlorinated compounds were acquired on a laboratory scale configuration in stop-flow and flow-through closed-loop modes, in order to study the direct response of the sensor to any arbitrary concentration change of the analytes. Subsequently, the bioreactor (a fixed bed which could also simulate a sediment matrix) was monitored with the infrared sensor coupled permanently to it. The sensor tracked the progression of the analytes' spectra over time without perturbing the dechlorinating process. The accuracy of this ATR-FTIR sensor was validated against gas chromatography (GC) measurements of the chlorocompounds[17].

1.6. CONCLUSION

Microbial processes play crucial roles in the transformation of organic and inorganic compounds in sediments. The recent developments in molecular biology allowed a better understanding of the distribution, diversity, and functions of microbes in sediments. Novel key processes have been attributed to microbes thanks to molecular biology such us anaerobic methane oxidation. Coupling these molecular techniques with geochemistry involving sophisticated tools such as chemical and biochemical sensors opens up interesting horizons for comprehensive studies of microbial processes in sediments.

1.7. ACKNOWLEDGEMENTS

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2. Accumulating basic knowledge on biodegradation processes and use of this knowledge for analyzing catabolic potential and metabolic networks *in situ* by culture-independent approaches

Various approaches to clean contaminated environments have been proposed including chemical, physical or biological treatments. Among them, the biological treatment is considered an efficient and cost saving way to achieve remediation of contaminated sites[19-21]. During the previous years, natural attenuation, "naturally occurring processes in soil and groundwater that act without human intervention to reduce the mass, toxicity, mobility, volume or concentration of contaminants in those media"[22] has received increasing attention. It is generally accepted that microorganisms are the principal mediators of the natural attenuation of many pollutants. They transform or mineralize pollutants, thereby usually decreasing their masses and toxicities, in contrast to most other processes contributing to natural attenuation. However, in some cases pollutants may be transformed into more toxic products, as reported for the anaerobic transformation of trichloroethylene. The use of natural attenuation thus requires a detailed monitoring to determine how effective natural attenuation is for attaining site remediation goals. In order to properly evaluate natural attenuation at a site, it is necessary to know the location and concentration of the contaminants, how the contaminants move in the environment, and how their concentration changes over time. Reliance on biologically meditated natural attenuation thus requires information on whether it can occur, whether it is actually occurring at a significant rate, which mechanisms and pathways are involved and, specifically, how it will behave in the future. Active *in situ* remediation involves the stimulation of the indigenous microbial activity by the addition of nutrients and/or electron acceptors. As in the case for natural attenuation, an understanding of the response of the indigenous microbial community is necessary for successful stimulation. Even more unpredictable are bioaugmenation approaches, as the added microorganisms, which are supposed to increase the degradative performance on site, not only have to express these capabilities under *in situ* conditions, but also have to compete with the complex microflora inhabiting the site under study. It is thus evident that only a detailed understanding of the functioning and interactions in microbial communities will allow their rational manipulation, and the overcoming of factors limiting efficient bioremediation.

2.1. BACTERIAL DEGRADATION OF AROMATIC POLLUTANTS

Aromatic compounds have been discharged into the environment during the last years in increasing amounts. They have significant impact on natural microbial communities, and thus, on the global element mass fluxes. The functional diversity in nature shows that many microorganisms have the potential to degrade and recycle aromatic compounds[23]. This potential can be used for the bioremediation processes mentioned above.

Many microorganisms have evolved biodegradative pathways to use aromatic compounds as a sole carbon and energy source[24-26]. Even though the first observations were made using aerobic microorganisms[27,28], it is well established that aromatics can be degraded under nitrate-, iron-, or sulfate-reducing and even under methanogenic conditions[29,30].

2.1.1. *Rieske non-heme iron oxygenases*

Aerobic microorganisms usually initiate degradation by activation of the aromatic nucleus through oxygenation reactions. The introduction of hydroxyl-groups, usually in *ortho*-position to one another, results in a few central intermediates such as catechols, protocatechuate, gentisate and hydroxyhydroquinones. These intermediates are subject to oxygenolytic ring cleavage followed by channeling of the ring-cleavage products into the central metabolism[31].

The so called Rieske non-heme iron oxygenases are one of the key families of enzymes important for aerobic degradation of aromatics. These enzymes usually catalyze the incorporation of two oxygen atoms into the aromatic ring to form arene-*cis*-dihydrodiols[32], a reaction which is followed by a dehydrogenation catalyzed by *cis*-dihydrodiol dioxygenases to give catechols or substituted catechols. The oxygenases, such as benzoate, naphthalene, biphenyl, or toluene dioxygenases are multicomponent enzyme complexes, composed of a terminal oxygenase component (iron-sulfur protein) and different electron transport proteins (a ferredoxin and a reductase or a combined ferredoxin-NADH-reductase). The catalytic iron-sulfur proteins are heteromultimers, comprising a large (α) and a small (β) subunit with the former containing a Rieske-type [2Fe-2S] cluster, a mononuclear non-heme iron and a substrate binding site (Fig. 2.1).

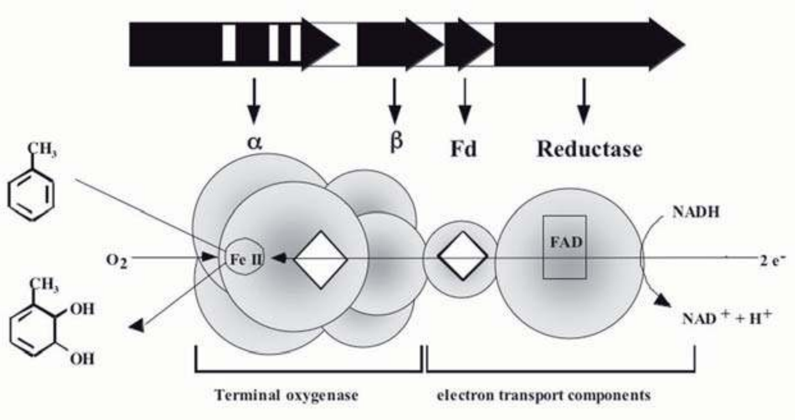

Figure 2.1. Biochemical and genetic organization of toluene 2,3-dioxygenases as an example of Rieske non heme iron oxygenases. Two electrons are transferred from NADH via the electron transfer chain, consisting of reductase and ferredoxin (Fd) to the terminal oxygenase component, comprising the small α-subunit and the large β-subunit. The incorporation of two oxygen atoms into the substrate results in the formation of a *cis*-dihydrodiol. Regions of the gene encoding residues of the α-subunit responsible for substrate specificity are indicated in white.

Comparison of the amino acid sequences of the terminal oxygenase α-subunits revealed that they form a family of diverse but evolutionarily related sequences (Figure 2.2). Although none of the enzymes is completely specific, a broad correlation between the grouping in toluene/biphenyl, naphthalene, benzoate or phthalate families and the native substrates oxidized by the family members can be observed.

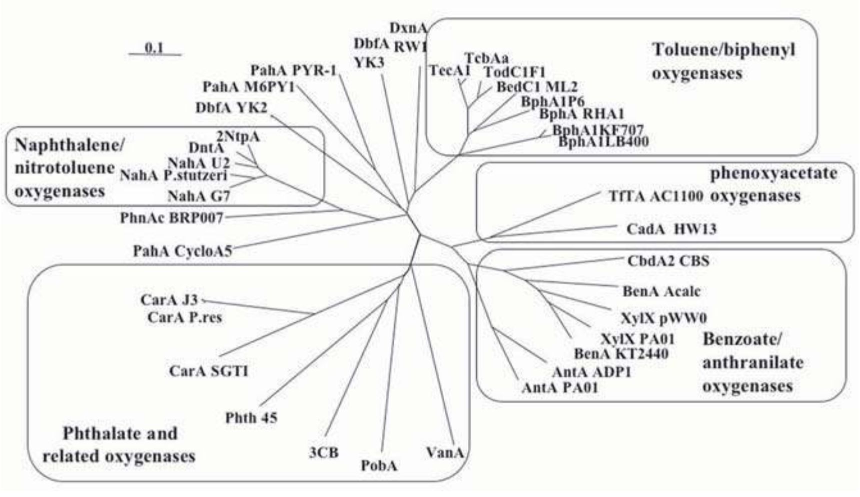

Figure 2.2. Dendrogram showing the relatedness of members of different families of Rieske non heme iron oxygenases (α-subunits).

Enzyme engineering studies on biphenyl, benzene, chlorobenzene, and naphthalene dioxygenases showed that the α-subunit of the terminal oxygenase determines substrate specificity and that only slight sequence differences in the amino acid sequence can be associated with dramatic changes in substrate specificity or regiospecificity[33-36]. As an example a single amino acid difference in toluene dioxygenase results in transformation of tetrachlorobenzene[33] or in a completely new regioselectivity of naphthalene dioxygenase[36]. Regioselectivity also determines whether or not a given substrate can subsequently be subject to mineralization. As an example, dibenzofuran can be mineralized after a so called angular attack at the oxygen bridge[37] and an adjacent carbon atom, whereas lateral attack results in the formation of dead-end products[38]. Consequently, dioxygenases crucially determine the range of substrates that are accessible to microbial degradation and the metabolic net in microbial communities. Crystal structures are available for some members of Rieske non-heme iron oxygenases[39,40]. Those allow the modeling of active site structures of newly discovered derivative enzymes and at least a partial explanation of enzyme performance, even though amino acid residues, which have no contacts with substrates also strongly changed enzyme performance[41,42]. Nevertheless, Rieske-type non-heme iron oxygenases are an enzyme family in which significant advances have been made to relate sequence information to function.

2.1.2. *Toluene monooxygenases*

Two groups of enzymes have been reported to attack the non-activated benzene nucleus by monooxygenation, the multicomponent aromatic monooxygenases and some members of the multicomponent phenol hydroxylases[43], both groups belonging to the family of soluble diiron monooxygenases. These monooxygenases can perform regio- and stereospecific hydroxylations (Fig. 2.3), and in the case of transformation of toluene, 2-methylphenol[44], 3-methylphenol[45] as well as 4-methylphenol[46], were reported to be formed, however, recent results showed that 4-methylphenol but not 3-methylphenol is formed by the enzyme termed toluene 3-monooxygenase[47]. Further oxygenation of the intermediate cresols by phenol hydroxylase results in the formation of 3-methyl- or 4-methylcatechol, respectively.

Alternatively, 4-methylphenol can be subject to oxygenation of the methylsubstituent resulting finally in protocatechuate (Figure 2.3) as ring-cleavage substrate[48]. Evidently, again, substrate specificity will significantly determine the metabolic net responsible for degradation.

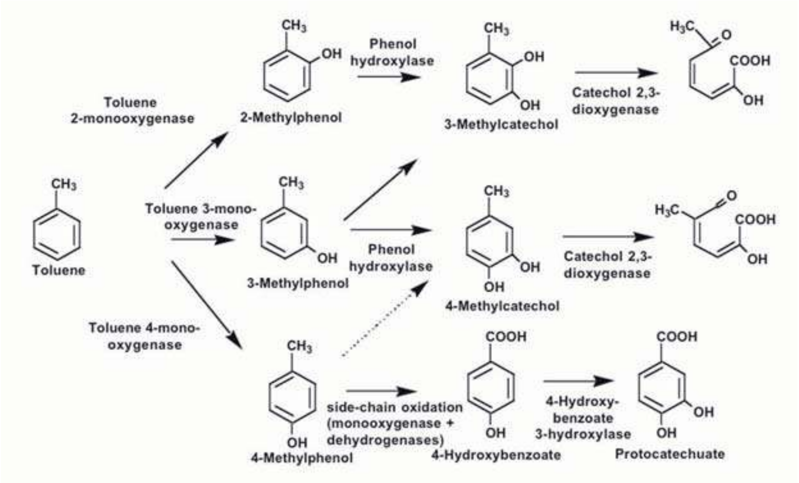

Figure 2.3. Transformation of toluene by two successive monooxygenations results in the formation of methylcatechols which are usually mineralized via extradiol cleavage.

Moreover, dependent on the regioselectivity, xylenes will be transformed to dead-end products. The aromatic monooxygenases consist of an electron transport system comprising a reductase and a ferredoxin, a catalytic effector protein which is assumed to play a role in assembly of an active oxygenase and a terminal hydroxylase with a $(\alpha\beta\gamma)_2$ quaternary structure and a diiron center contained in each α-subunit[43]. Even though the effector protein is necessary not only for effective coupling, but also for a high regioselectivity[49], the α-subunit was majorly responsible for substrate specificity and regioselectivity[43]. Various mutagenesis and directed evolution studies have actually identified residues which control regioselectivity and activity[50,51]. The structure of toluene monooxygenase from *P. stutzeri* OX1 has recently been elucidated[52] which offers the opportunity to understand how the enzyme adjusts the active site pocket to control product regioselectivity.

2.1.3. *Catechol dioxygenases*

Whereas a relatively broad diversity of activation mechanisms is possible in aromatic degradation, these pathways usually converge in the formation of (substituted) catechols as central intermediates[53]. Whereas chlorinated aromatics are predominantly degraded via intradiol cleavage[54] methylsubstituted aromatics such as toluene or xylenes are usually degraded via the respective catechols and extradiol cleavage (Fig. 2.3). Extradiol dioxygenases are thus key enzymes in the degradation of aromatic compounds and many of such proteins and their coding sequences have been described, purified and characterized in the last decades. Examination of their evolutionary relationships[55] showed that the majority of extradiol dioxygenases with preferences for monocyclic substrates were, at the protein level, phylogenetically closely related. However, members of this enzyme subfamily were reported to differ significantly in their substrate specificity[42,56] and, thus, the metabolic network in environmental communities will be significantly interconnected with the C23O diversity. Catechol transforming activities are specifically important, as catechols are highly toxic for microorganisms[57] and even low accumulation will have severe impact in community functioning[58].

2.1.4. *Anaerobic degradation of aromatics*

In recent years our knowledge on anaerobic degradation of aromatics has been significantly enhanced and genetic determinants for various key steps in anaerobic aromatic degradation have been elucidated. Under anaerobic conditions, aromatic hydrocarbons are initially attacked by novel reactions and in most cases oxidized further to benzoyl-CoA, a common intermediate in anaerobic catabolic pathways[29]. Toluene degradation is initiated by an unusual addition reaction of the toluene methyl group to the double bond of fumarate to form benzylsuccinate[59] catalyzed by benzylsuccinate synthase, a unique type of glycyl-radical enzyme[60]. However, not only toluene degradation is initiated by fumarate addition. Similar reactions have been shown to be involved in the anaerobic activation of *m*-xylene, *m*- and *p*-cresol, *n*-alkanes and 2-methylnaphthalene[61,62]. Even though the metabolism of toluene has mainly been elucidated in denitrifying *Thauera* and *Azoarcus* it became evident that benzylsuccinate forming activities are found across a wide range of phylogenetically and physiologically diverse bacteria, and have also been found in the iron-reducing *Geobacter metallireducens* strains[63]. In contrast to the information on degradation of toluene and xylenes, information on anaerobic degradation of naphthalene and benzene is scarce[64,65].

2.1.5. *Reductive dehalogenation*

It is known since more than one decade ago that chloroaromatics can function as an alternative electron acceptor in a type of anaerobic respiration[66]. Several anaerobic bacteria have been identified as being able to reductively dehalogenate chlorinated aliphatics as well as chlorinated aromatics and to couple this reaction to the synthesis of ATP via a chemiosmotic mechanism. Bacterial dehalorespiration is currently considered to be the most important process for the detoxification of organohalogens under anaerobic conditions. The dehalorespiring bacteria known to belong to the low GC content Gram-positive bacteria (*Desulfitobacterium* and *Dehalobacter*), the Proteobacteria (*Desulfomonile*, *Desulfuromonas* and *Dehalospririllum*, now transferred to the genus *Sulfurospirillum*), and the genus *Dehalococcoides*[67]. Although dehalorespiring bacteria are widespread, *Dehalococcoides* species in particular seem to be of major environmental importance. It has been shown that members of this group can grow on vinyl chloride as electron acceptor[68], a process previously assumed to be co-metabolic (i.e. without deriving energy or carbon from the transformed substrate). In addition, *Dehalococcoides* strains are, thus far, the only microorganisms known to be capable of growing anaerobically on chlorobenzenes[69], polychlorinated biphenyls and even chlorinated dioxins[70]. Reductive dehalogenases involved in tetrachloroethene or chlorophenol respiration have been intensively studied (Figure 2.4). This novel subclass of reductases shares some common features at the biochemical and genetic level. Except for the chlorobenzoate reductive dehalogenase of *Desulfomonile tiedjei* that contains a heme cofactor and that is produced as a heterodimer[71], reductive dehalogenases contain two iron–sulfur clusters and a corrinoid[67].

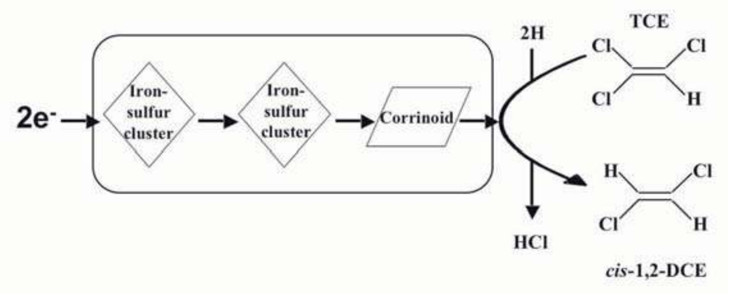

Figure 2.4. Reductive dehalogenation of TCE (trichloroethene) to *cis*-1,2-dichloroethene.

They are produced as preproteins with a twin-arginine signal peptide recognized by the export system specific for the export of periplasmic proteins containing cofactors[72]. The protein sequence also revealed consensus sequences characteristic for the binding of two iron–sulfur clusters. Also vinyl chloride reductive dehalogenase was recently identified as a novel member of the family of corrinoid/iron-sulfur cluster containing reductive dehalogenases[73].

2.2. MOLECULAR TOOLS FOR ASSESSING BIODEGRADATION POTENTIAL

It has been shown that the *in situ* catabolic potential of microbial communities depends on the history of the site under study, the site characteristics (geochemistry and redox conditions) and, in cases where the contaminated soil is covered by vegetation, interactions between the microbial community and plant species, which are up to now poorly defined. Moreover, it is well known that many environmental microorganisms may not be cultivated under laboratory conditions. Culturing-independent molecular techniques are, currently, rapidly increasing our understanding of microbial community structure and activity in the subsurface. Polymerase Chain Reaction (PCR) amplification of nucleic acids extracted from environmental samples is at present the most powerful cultivation-independent technique. PCR facilitates the sensitive and fast detection of low amounts of specific gene fragments. This is of importance for monitoring purposes, as subsurface environments are, in general, oligotrophic, hence characterized by low biomass from which low amounts of nucleic acids can be extracted.

Molecular microbial ecology has significantly increased our knowledge on the diversity and dynamics of microbial communities in nature. By different PCR approaches, predominantly using the 16S rRNA gene or the inter-spacer region of 16-23S rRNA genes, specific taxonomic groups responsible for degradation, and specific species, can be characterized, quantified and their spread followed over time[74]. However, as only in a subset of scenarios, a degradation capability is directly related to a specific taxonomic group, research in recent years has focused on the adaptation of molecular ecology methods for assessing community composition to characterize the makeup of catabolic genes, as the abundance of degradation genes can be assumed to indicate the biodegradation potential in contaminated soils.

2.2.1. *Analysis of catabolic genes*

Catalytic enzymes involved in aromatic degradation are encoded by genes, which have evolved to produce proteins performing specific actions. Members of the different gene families share a certain degree of similarity represented by conserved sequence regions. With only two short sequences conserved along two fairly divergent gene family members, it is possible to detect, by amplification, regions between the conserved parts, in DNA from isolates or from environmental samples. The initial step in this process is to identify the crucial gene and protein regions. Subsequently, conserved regions are identified within the different groups of given gene families and evaluated for primer design.

Thus far, analysis of catabolic genes has focused mainly on the characterization of the presence or abundance of a family of catabolic genes[73,75,76] without assessing the finer levels of sequence diversity. However, the diversity of catabolic gene sequences as described above, often reflects differences in substrate specificity or affinity. Single amino acid differences have been reported to drastically change substrate specificity of catabolic enzymes (see above). A more detailed picture of the catabolic gene structure and sequence diversity in environmental samples will, thus, significantly increase our knowledge of the functional potential of microbial communities. Moreover, shifts in catabolic gene structure will allow the deduction of the evolutionary fitness of catabolic genes, operons and their respective hosts. A variety of molecular fingerprinting approaches previously developed to assess community structure, via the analysis of 16S rDNA or rRNA diversity, may be applied to define functional gene structure and we succeeded recently[77] to adapt fingerprinting methods to elucidate the diversity of various key families of catabolic genes. Together with promising methods to characterize functional gene expression in environmental samples, and an improved knowledge on structure/function relationships in catabolic genes high throughput fingerprinting methods would help future trends to identify catabolic gene diversity and structure, as well as active operons and pathways under changing environmental conditions.

2.2.2. *Fingerprinting of catabolic genes*

A promising fingerprinting technique that is able to resolve fragments of identical length but different sequence composition is SSCP (single strand chain polymorphism). This technique (Figure 2.5) takes advantage of the property of the sequence-dependent conformation acquired by single-strand DNA molecules separated in gels under non-denaturing conditions. The application was originally conceived to compare amplifications from single templates to rapidly identify single nucleotide polymorphisms in the spanned sequence fragment[78]. For each single amplification (of a dsDNA PCR product) two single strands are produced generating two different conformations. The SSCP method was optimized to discriminate sequence diversity of environmental 16S rRNA genes by amplification with one 5’end phosphorylated primer, allowing the degradation of the phosphorylated strands of each PCR amplicon by lambda exonuclease digestion. This simplifies and improves the resolution of the method for complex amplicon mixtures[79]. The SSCP-approach has been used in various studies identifying changes in predominant members and bacterial taxonomical composition[80].

Because of their central role in aromatic catabolism, the catechol 2,3-dioxygenases (C23O) subfamily I.2.A genes have been analysed in studies of diverse environments and their presence has been observed in various contaminated sites[75,81].

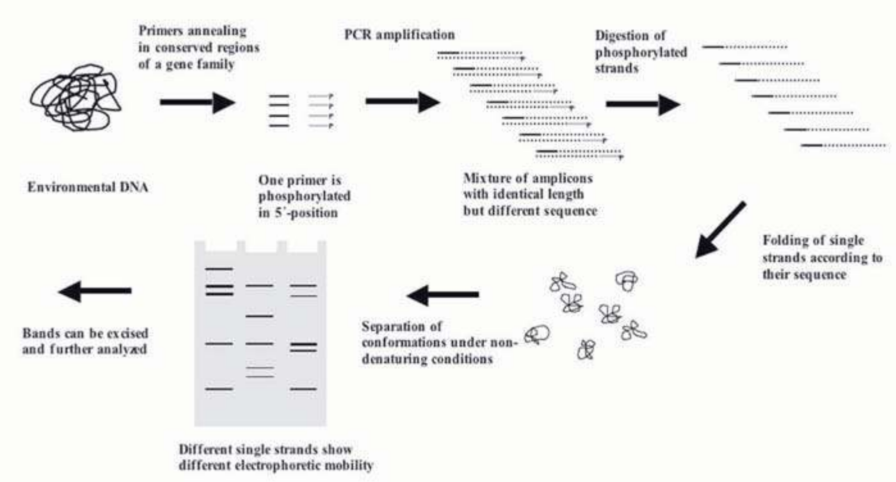

Figure 2.5. Schematic representation of the PCR-SSCP fingerprinting technique, which can be used to analyze complex amplicons from environmental DNA.

Genes encoding catechol 2,3-dioxygenases (C23O) were used as functional targets to assess the catabolic gene diversity in differentially BTEX (benzene, toluene, ethylbenzene, xylene) contaminated environments by polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP)[77]. Site specific PCR-SSCP fingerprints were obtained, showing that gene diversity experienced shifts correlated to temporal changes and levels of contamination. Overall, the PCR-SSCP technique was shown to be a powerful tool for assessing the diversity of functional genes and the identification of predominant gene polymorphs in environmental samples as a prerequisite to understand the functioning of microbial communities. PCR-SSCP profiling of a defined catabolic gene family with limited diversity lower the possibility of different single strands having identical mobility, in contrast to the use of PCR-SSCP for detection of highly diverse 16S rRNA gene sequences where bands were found to contain a multitude of different sequence types[80].

As described above, Rieske non-heme iron oxygenases are a key family defining aerobic degradation networks. The same contaminated site and various isolates were used to develop a SSCP fingerprinting method covering regions defining substrate specificity and gene variants with new active site structure could be observed to be predominant in contaminated sites and obviously selected for by benzene as contaminant (Witzig, unpublished).

2.3. NEW METABOLIC ROUTES OBSERVED IN ISOLATES

The above described methods rely on nucleic acid probes and PCR primers designed on the base of information retrieved from isolates and can thus only cover a subset of the activities assumed to be present in environmental samples and will not cover new genes or gene products. However, even isolate-based approaches still recover a broad set of new metabolic diversity previously undescribed.

One example is a new pathway for aerobic aromatic metabolism initially observed in *Azoarcus evansii*[82,83]. It could be shown that in various bacteria benzoate is first converted to its coenzyme A thioester, benzoyl-CoA, which is subsequently attacked by an oxygenase, followed by a non-oxygenolytic ring-fission. Respective genes were also observed during sequencing of the genome of the biphenyl degrading organism *Burkholderia xenovorans* LB400 and recently it could be shown that benzoate, when produced during biphenyl metabolism by this strain, is actually degraded via the benzoyl-CoA pathway[84].

Also new routes for the degradation of chlorocatechols as central intermediates in aerobic chloroaromatic degradation could be elucidated, which ressemble variations of or patchworks between archaetype catechol degradation pathways and chlorocatechol pathways. In *Rhodococcus opacus* 1CP, the dehalogenation reaction during 3-chlorocatechol degradation is not catalyzed during cycloisomerization, as described for chlorocatechol pathways, but by a specialized muconolactone isomerase similar to enzymes commonly involved in catechol degradation[85]. 4-Chlorocatechol degradation by *Pseudomonas* sp. MT1 is initiated by enzymes resembling those of the archaetype catechol degradation pathway, however, dehalogenation is catalyzed by a new type of hydrolase acting on an unstable pathway intermediate[86]. Recent sequencing of the gene revealed that the protein does not show significant sequence similarity to any gene of described function available in public databases (Cámara, unpublished).

2.4. METAGENOMICS

One approach that relies neither on conserved nucleotide sequences nor on previous knowledge on isolates is the use of genomic libraries to retrieve genes from natural bacterial communities without cultivation. The field of metagenomics is currently rapidly developing. Genomic DNA is extracted and inserted into vectors, such as plasmids, cosmids or bacterial artificial chromosomes, which can maintain inserts of around 100 kb. These can then be propagated in appropriate bacterial strains, usually *E. coli* and be screened for expressed catabolic activities. Interestingly, the activity-based screening of metagenome libraries usually resulted in the discovery of gene products, which were only distantly related to previously described ones[87,88]. However, there are two significant drawbacks of the current procedures: (a) the necessity to screen an immense set of clones, only a few of which encode the desired activity, especially if the screening system is time consuming, and (b) the poor expression in *E. coli* of many genes present in metagenome libraries. In an effort to quantify the accessibility of the metagenome, it was shown that only 40% of the genes present in the genome of 32 prokaryotes could be easily detected in *E. coli*[89]. However, new *Streptomyces* and *Pseudomonas* strains that are optimized to express environmental gene libraries have recently been constructed[90]. One approach to overcome time-consuming screening assays is a procedure termed substrate induced gene expression screening[91] which is based on the knowledge that catabolic gene expression is generally induced by substrates and metabolites of catabolic enzymes and, in many cases, controlled by regulatory elements situated proximate to catabolic genes. A metagenomic library was constructed in a green fluorescent protein (GFP)-expression vector, and clones expressing GFP in the presence of a target substrate were selected by cell sorting. By this procedure, novel catabolic genes could successfully be isolated from a metagenome library.

Considering the well-known limitation to describe microbial diversity by culture-dependent approaches, the analysis of environmental metagenomes will probably reveal that actually only a fraction of the metabolic capacity of microorganisms is thus far documented. This also holds for the metabolic capacity to degrade aromatic pollutants. Specifically the mechanisms of anaerobic biodegradation need to be characterized and exploited to their finer details, as it has been done in the studies for aerobic degradation pathways, where more detailed biochemical and genetic information has been obtained thus far.

2.5. ACKNOWLEDGEMENTS

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3. Detection and characterization of microbial processes associated to PCB degradation in marine contaminated sediments

PCBs are poorly biodegradable and highly toxic contaminants. Due to their high hydrophobicity, PCBs released into aquatic systems tend to strongly accumulate in anoxic freshwater, estuarine and marine subsurface sediments[92], where half-lives of several months up to many years have been estimated. Several studies have documented the occurrence of reductive dechlorination processes towards PCBs in anaerobic freshwater sediments, where highly chlorinated *meta* and *para*-substituted PCBs can be generally bioconverted, mainly under methanogenic conditions, into low-chlorinated *ortho*-substituted congeners[93,94]. On the contrary, little is known about the occurrence of reductive dehalogenation processes towards weathered PCBs in marine sediments[95,96], where in general sulfidogenic conditions prevail over methanogenesis. To our knowledge, the reductive dechlorination of pre-existing PCBs was documented only once in sediments of New Bedford Harbor under methanogenic conditions[95,97]. Some other studies have documented the reductive dechlorination of PCBs in sediment slurries developed with salt rich media where, however, spiked PCBs and/or synthetic media were employed[98,99,100]. Thus, more information on the potential fate of aged PCBs in marine contaminated sediments is required. In particular, we need studies performed on real contaminated sediments suspended in their own real water under laboratory conditions that closely mimic those occurring *in situ*[93,94] .This might allow us to collect information of some relevance for predicting, when combined to lines of biogeochemical evidence[101], the potential of biological processes in the final *in situ* restoration of contaminated sediments. In this paper, the main results are reviewed of two studies in which the occurrence of microbially mediated reductive dechlorination processes towards aged and spiked PCBs has been detected and characterized in distinct sediments of the Porto Marghera area (Venice lagoon, Italy) suspended in water from the site. Preliminary data on the dechlorination activity and the microbial composition of a consortium enriched from the most active microcosms are also presented.

3.1. FIRST DETECTION AND CHARACTERIZATION OF REDUCTIVE DECHLORINATION PROCESSES IN THE SEDIMENTS OF THE VENICE LAGOON

3.1.1. *Experimental approach*

A series of slurry-phase anaerobic microcosms consisting of a PCB contaminated sediment of the industrialized area Porto Marghera (Brentella Canal, Venice lagoon, Italy) suspended at 25% (v/v) in the water collected from the same contaminated area were developed. Replicate microcosms were set up also under different conditions in order to select for various indigenous microbial populations and to indirectly determine their potential involvement in PCB biotransformation. Pasteurized microcosms, as well as molybdate-amended and 2-bromoethanesulfonate (BES)-amended microcosms, were established in order to select for spore-forming bacteria, to inhibit sulfate-reducing bacteria and to inhibit methanogenic bacteria, respectively. In addition, sterile microcosms were set up under each condition. Finally, a parallel set of identical microcosms was also spiked with 2,3,4,5,6-pentachlorobiphenyl (20 mg/kg dry wt sediment) in order to study the possibility of "priming" the dechlorination of the sediment aged PCBs and the mechanism through which they were dechlorinated under each of the experimental conditions. Microcosms were set up in 30 ml serum bottles starting from a preliminary sediment slurry (25% v/v) that was prepared under strictly anaerobic conditions as described by Fava et al.[102]. BES and molybdate were added as stock solutions in sterile lagoon water to the final concentration of 30.0 mM and 20.0 mM, respectively. Six and a half μl of an acetone stock solution (70 mM) of 2,3,4,5,6-pentachlorobiphenyl were added to each spiked microcosm under sterile conditions to yield a final concentration of about 20 mg (kg dry wt sediment)$^{-1}$, whereas the same volume of fresh acetone was added to the non-spiked microcosms. Pasteurization and sterilization were performed before adding the exogenous PCB. Pasteurized microcosms were obtained with a 15 minutes treatment at 90°C in a water bath, whereas sterile microcosms underwent autoclaving treatment at 121°C for 1 h for 3 consecutive days with incubation at 28°C between each autoclaving treatment[102]. Under each culture condition, a set of triplicate biologically active and sterilized microcosms was prepared. All the developed microcosms were then incubated stationary at 25 ± 1°C in the dark for 20 weeks. During this period they were periodically sampled and analyzed to determine the volume and the composition of the headspace gas, as well as the concentration of PCBs and inorganic anions (i.e., $SO_4^{=}$ and Br^-) according to Fava et al.[102].

At the end of the experiment the Terminal-Restriction Fragment Length Polymorphism (T-RFLP) technique was used to investigate the complexity of the microbial population in the most active microcosms as compared to the original sediment. The DNA was extracted from 0.6 grams of original sediment (conserved at 4°C in the dark under strict anoxic conditions) and from the pellet of 1.5 ml of slurry according to Scala and Kerkhof[103]. DNA was purified by CsCl gradient and 16S rDNA was amplified using universal bacterial [6]-carboxyfluorescein-labelled 27F (5'-AGAGTTTGATCM-TGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCAR-3') primers by Polymerase Chain Reaction (PCR). Five µl of the PCR products were then digested at 37°C for 2 hours with *MnlI*, *RsaI* and H*haI* in three independent 20 µl reactions, precipitated and resuspended in 14.75 µl of deionized formamide and 0.25 µl of TAMRA 500 (Perkin-Elmer, Boston, MA, USA). Fluorescently labelled fragments were then separated and detected with an ABI Prism 310 capillary sequencer (PE Applied Biosystem, Foster City, CA, USA) using the GeneScan mode. T-RFLP profiles were compared by Sorensen's similarity analysis.

3.1.2. *Results and discussion*

A total PCB concentration of 0.784 ± 0.351 μmol (kg dry wt sediment)$^{-1}$ was detected in the biologically-active and sterile microcosms at the 7th day of incubation. Significant changes of the initial PCB distribution profile were unequivocally observed in the untreated microcosms at the 20th week, where an extensive depletion of highly chlorinated biphenyls together with a stoichiometric accumulation of tri- and di-chlorinated, *ortho*-substituted biphenyls were observed (Fig. 3.1A).

A less extensive but significant transformation of endogenous PCBs was also observed in the pasteurized microcosms, where the accumulation of 2-chlorobiphenyl was also observed (Fig. 3.1B). On the contrary, the sediment PCBs were only poorly transformed in the microcosms supplemented with BES or molybdate (Figs. 3.1C and 3.1D, respectively). Comparable changes in the endogenous PCBs distribution profiles were observed in the 2,3,4,5,6-pentachlorobiphenyl-spiked microcosms[102].

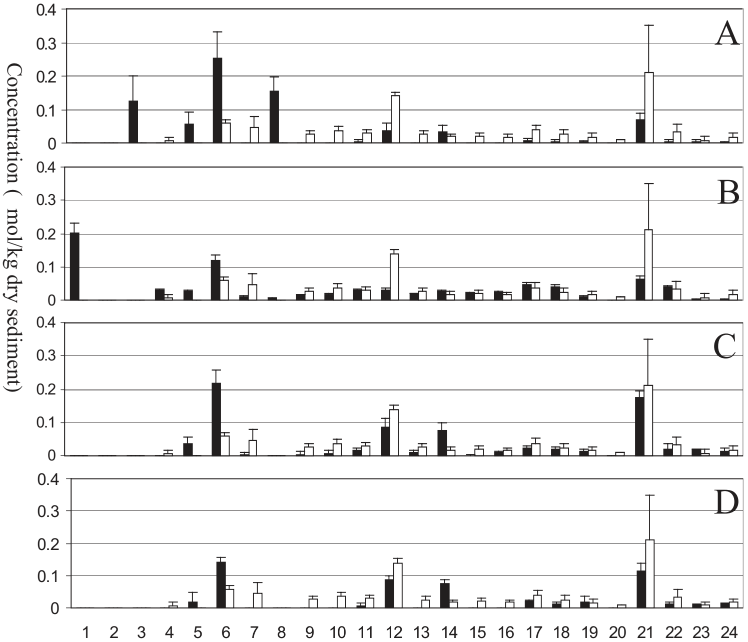

Figure 3.1. Bioconversion of sediment-carried PCBs after 20 weeks of incubation. White bar: sterile microcosms; Black bar: biologically active microcosms. A: untreated microcosms; B: pasteurized microcosms; C: BES amended microcosms; D: Molybdate amended microcosms. (1):2-CB; (2):4-CB; (3):2,6-/2,2'-CB; (4):2,4-/2,5-CB; (5):2,4'-/2,3-CB; (6):2,4,6-CB; (7):2,2',5-/2,2',4-/4,4'-CB; (8):2,3,6-/2,3',6-CB; (9):2,3,3'-/2',3,4-/2,2',5,6'-CB; (10):2,2',4,6'-/2,3,4'-CB; (11):2,2',5,5'-CB; (12):3,3',4-CB; (13):2,2',3,5-CB; (14):3,4,4'-/2,3,3',6-/2,2',3,4'-CB; (15):2,2',3,4-/2,3,4',6-CB; (16):2,3',4',5-CB; (17):2,3',4,4'-/2,2',3,5',6-CB; (18):2,2',3,4',5-/2,2',4,5,5'-CB; (19):2',3,4,4',5-/2,2',3,4',5',6-/2,3',4,4',5-CB; (20):2,2',-3,4',5,5'-CB;(21):2,2',3,3',4,6'-/2,2',4,4',5,5'-/2,3,3',4,4'-CB;(22):2,3,3',4,5,6-/2,2',3,4,4',5/-2,3, 3'4,4',6-CB; (23):2,2',3,3',4,5,6-/2,3,3',4,4',5'-/2,2',3,3',4,5',6,6'-CB; (24):-2,2',3,4,4',5,5'-CB. CB: chlorobiphenyl.

A detectable production of gas was observed in all the biologically active microcosms during the 20 weeks of incubation (Table 3.1). In the molybdate-amended microcosms a significant methane production and no consumption of the initial 2.1±0.1 g l^{-1} of $SO_4^{=}$ were observed (Table 3.1). On the contrary, a consumption of about 30-50 % of the initial $SO_4^{=}$ was observed in the untreated, pasteurized and BES-amended microcosms, where no methane production was detected. In addition, in the BES-amended microcosms a release of about 6 mg l^{-1} of Br^- was measured (Table 3.1). The same changes were observed in the PCB-spiked set of microcosms, except for sulfate consumption in the untreated microcosms, where it was slightly faster and more extensive than in the corresponding non-spiked microcosms (data not shown)[102].

Table 3.1. Microbial activities detected in the microcosms after 20 weeks of incubation.

Microcosms	Sulfate depletion (%)	Produced Gas (ml)	Produced CH_4 (ml)	Released Br^- (mg/l)
Sterile microcosms	-19.3 ± 9.3	0	0	0
S + W**	29.9 ± 7.8*	1.25 ± 0.21	0	0
S + W, Pasteurized	41.2 ± 14.2*	0.65 ± 0.21	0	0
S + W + Molybdate	-12.4 ± 2.7*	2.40 ± 1.00	0.17 ± 0.01	0
S + W + BES	47.3 ± 35.0*	1.55 ± 0.64	0	6.01 ± 0.88

*: values corrected for the percentage sulfate consumption in the sterile microcosms.

**: S + W = sediment + water

Taken together, these results indicate that indigenous sulfate-reducing bacteria were responsible for the detected PCB-reductive dechlorination. A low dechlorination activity was detected in the microcosms supplemented with BES, where a marked consumption of $SO_4^{=}$ was observed. A significant release of Br^- was also observed in these microcosms; this suggests that BES acted as the preferential electron acceptor for the dehalogenating bacteria, thus inhibiting PCB dechlorination. The detection of both reductive dechlorination activity and sulfidogenic activity in the pasteurized microcosms indicates that spore-forming, sulfate-reducing bacteria were involved in the process. A similar hypothesis has already been proposed in the literature[93] but it is the first time that it is formulated for the reductive dechlorination of PCBs pre-existing in a marine sediment resuspended in the site water. The exogenous 2,3,4,5,6-pentachlorobiphenyl was markedly bioconverted into its tetra-, tri- and di- *ortho*-chlorinated daughter products in the spiked untreated microcosms, showing that the process had a higher selectivity towards *meta*- and *para*- positions. A less extensive but significant 2,3,4,5,6-pentachlorobiphenyl transformation also occurred in the pasteurized microcosms, where 2-chlorobiphenyl was accumulated under these culture conditions, as also observed in the corresponding non-spiked microcosms[102]. Finally, the dechlorination of the spiked PCB did not significantly affect the onset of the pre-existing PCB dechlorination, probably because of the low concentration (≈ 1 mg/kg) of the weathered PCBs in the sediment and/or the inhibition by high concentrations of $SO_4^{=}$ and salt occurring in these microcosms.

At the end of the 20 weeks incubation, the spiked microcosms that exhibited the higher PCB dechlorination activity, i.e. the untreated and the pasteurized ones, were chosen for the analysis of the indigenous microbial population considering that the exogenous PCB may have favored the growth of PCB-dechlorinating microorganisms. For comparison, the analysis was also performed on the original sediment. Since different microbial taxons can be distinguished by the sequence of the 16S rRNA genes, we analyzed the polymorphisms of these genes using the T-RFLP technique. Three different restriction enzymes were chosen (*MnlI*, *RsaI* and *HhaI*) in order to better resolve the complexity of the microbial populations. Very different T-RFLP profiles were generated by the microcosms and the sediment (Fig. 3.2 for *MnlI* digestion), indicating that large modifications in the microbial community structure occurred in both the microcosms as compared to the original sediment. Sorensen's similarity analysis showed that the microbial population occurring in the untreated microcosms at the end of the experiment was identical to that detected in the original sediment only by the 26%, 29% and 32% when restriction digestion was performed with *MnlI*, *RsaI* and *HhaI*, respectively. A lower similarity was found between the microbial population detected in the pasteurized microcosm and in the sediment (8%, 10% and 25%, respectively). This low similarity is due to the higher complexity of the microbial population occurring in the microcosms, as revealed by the larger number of terminal fragments detected in these samples. The analysis of the relative abundance of each terminal fragment also showed that three major fragments were generated from the sediment DNA with each restriction enzyme. These were not detected or were detected at much lower level in the microcosms as compared to the sediment (Fig. 3.2 for *MnlI* digestion). In addition, many of the major peaks detected in the microcosms were not found or were poorly represented in the original sediment.

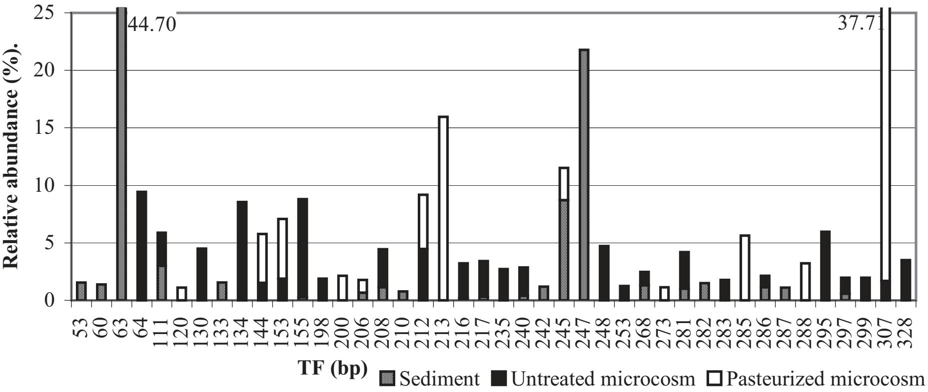

Figure 3.2. T-RFLP profile resulted from *Mnl*I digestion: percentage relative abundance of each terminal fragment calculated as peak area/total area ratio.

These data indicate that a selective enrichment of some microbial populations potentially involved in PCBs dechlorination occurred in both microcosms, probably as a consequence of the ongoing dechlorinating process towards PCBs. We cannot exclude the possibility that the observed effects could be also partly due to other factors, such as the incubation temperature, that could have favored indigenous mesophilic bacteria, or the sediment slurry mixing provided during the microcosms setup and sampling, that probably increased the bioavailability of organic substrates and sulfates. In addition, some components of the microbial population not detected in original sediment could have been derived from the site water used to prepare the slurry.

In conclusion, an extensive reductive dechlorination process towards pre-existing and spiked PCBs was observed in both untreated and pasteurized microcosms of sediment and site-water of the Venice lagoon. PCB dechlorination was selectively directed to *meta*- and *para*- positions and seemed to be mediated by sulfate-reducing and spore-forming indigenous bacteria. Finally, T-RFLP analysis showed that some microbial populations originally occurring in the sediment significantly enriched in the PCB dechlorinating microcosms. This could be the consequence of the specific environmental conditions established in the microcosms (e.g., an incubation temperature higher than that occurring at the site from which the sediment was collected, a good slurry homogeneity, that might have increased the bioavailability of sediment nutrients, etc.) but also to the PCB-reductive dechlorination processes that might have favored the specialized bacteria over the other indigenous ones.

3.2. REDUCTIVE DECHLORINATION PROCESSES OF COPLANAR PCBs IN ANOTHER SEDIMENT OF THE VENICE LAGOON AND PRELIMINARY CHARACTERIZATION OF ITS MICROBIAL POPULATION

3.2.1. *Experimental approach*

The sediment employed in this second work was also collected from the Brentella Canal. It was black, silty mud and contained approximately 1.6 mg/kg (on dry wt basis) of a mixture of PCBs which could be partially ascribed to PCBs of Aroclor 1242 and Aroclor 1254. A set of 8 slurry-phase anaerobic microcosms consisting of the sediment suspended at 25% (v/v) in water of the same contaminated area was developed under N_2:CO_2 (70:30) atmosphere according to Fava et al.[96]. Four of them (2 biologically active and 2 autoclave-sterilized) were used to investigate the occurrence of reductive dechlorination processes towards sediment-carried PCBs whereas the other 4 microcosms (2 biologically active and 2 autoclave-sterilized) were spiked with 3,3',4,4'-tetrachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl, 2,3',4,4',5-pentachlorobiphenyl, 3,3',4,4',5,5'-hexachlorobiphenyl and 2,3,3',4,4',5-hexachlorobiphenyl (all from Ultra Scientific, Rhode Island, USA) in order to investigate: a) the possibility of "priming" (i.e. stimulating) the dechlorination of PCBs pre-existing in the sediment, and b) the potential biological fate of target coplanar dioxin-like PCBs under the geochemical conditions created in the microcosms. Each exogenous PCB was individually added (as solutions at 10,000 mg/l prepared in acetone) to a final concentration of 100 mg/kg of dry sediment; a volume of pure acetone identical of that employed to provide PCBs in the spiked microcosms was added to the parallel non spiked microcosms. Sterile microcosms were prepared through autoclave-sterilization performed at 121°C for 1 h in three consecutive days; in the case of spiked control microcosms, exogenous PCBs were added after autoclave sterilization.

The developed microcosms were then incubated stationary at 25 ± 1°C in the dark for 16 months, during which they were periodically sampled and analyzed to determine the volume and the composition of the head-space gas, as well as the concentration of PCBs and of $SO_4^{=}$ according to Fava et al.[96].

3.2.2. *Results and discussion*

A total amount of sediment carried PCBs corresponding to 1.60 ± 0.13 mg/kg of dry sediment was found to occur in the non-spiked sterile and biologically active microcosms after 7 days of microcosm incubation. No transformation of such PCBs was detected in the sterile microcosms until the end of the experiment. On the contrary, after a 5 months lag phase a significant dechlorination activity towards pre-existing PCBs was detected in the corresponding non spiked biologically active microcosms. The reductive dechlorination process was directed towards several hexa-, penta- and tetra-chlorinated congeners, that were significantly depleted and stoichiometrically bioconverted into less chlorinated congeners at the end of the 16 months experiment. 2,2’,5/2,2’,4/4,4’-chlorobiphenyl and 2,4/2,5-chlorobiphenyl, as well as 4-monochlorobiphenyl, were the dechlorination products more significantly accumulated in the biologically active microcosms at the end of the experiment (Fig. 3.3).

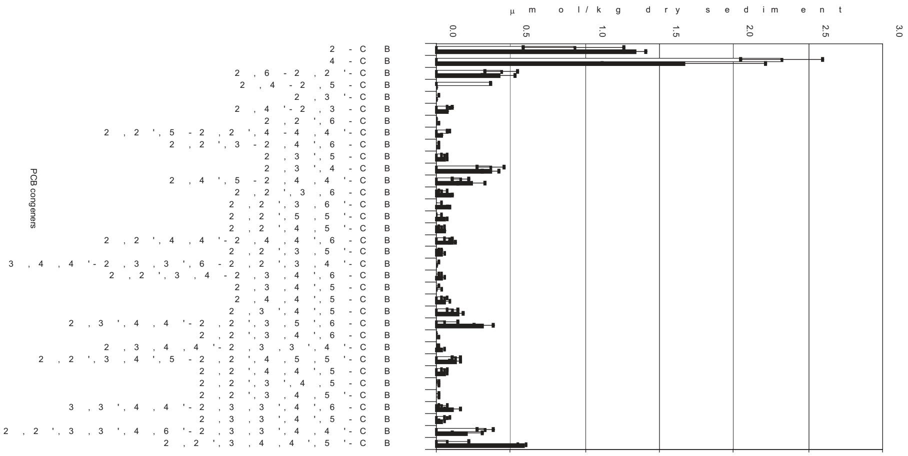

Figure 3.3. Changes in the PCB concentration in the biologically active non spiked microcosms (estimated vs. the sterile ones) at the end of the 16th month of incubation.

A detectable sulfate-reduction activity started to take place in the active microcosms since the first month of incubation. Sulfate was then quickly depleted by about 92% after 2 months of incubation and by 100% at the end of the 3rd month of experiment (Fig. 3.4). No significant biogas production was observed in the active microcosms until sulfate was completely depleted. A large amount of biogas (27.5 ± 16.5 ml) consisting of more than 47% of methane was detected in the same microcosms between the 3rd and the 5th month of incubation, i.e. immediately after complete sulfate depletion and before PCB dechlorination started. Methane production was detected at a lower rate over the whole experiment (up to the 16th month) (Fig. 3.4).

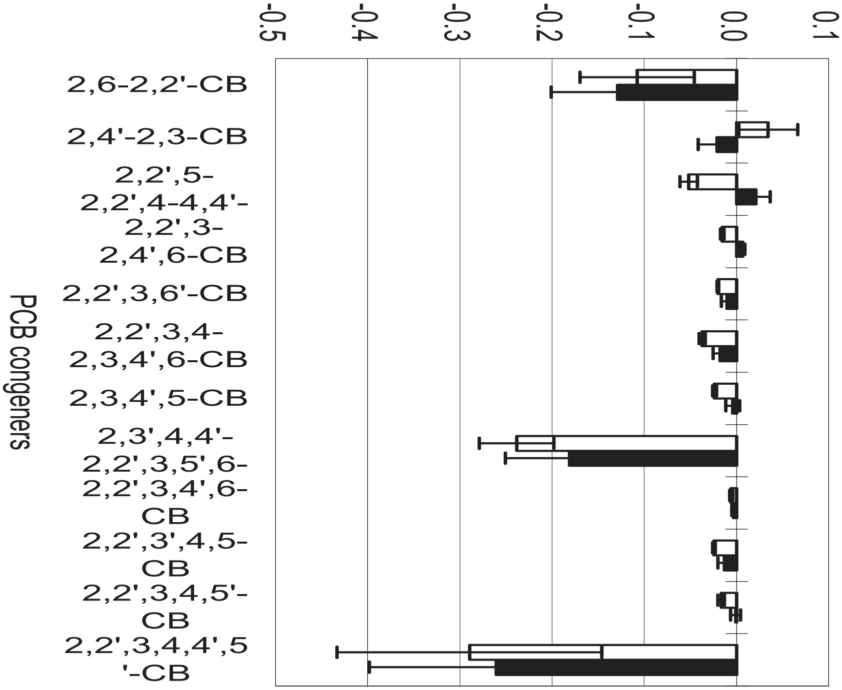

Figure 3.4. Sulfate consumption (solid line, empty symbols), biogas (solid line, solid symbols) and methane production (dashed line, solid symbols) in the biologically active non spiked (squares) and spiked (triangles) microcosms during the 16 months of incubation (standard deviation as error bars).

Very similar trends, both in terms of sulfate consumption and methane production, were observed in the parallel biologically active spiked microcosms, where the overall amount of produced methane was about 60% of that detected in the non spiked ones (Fig. 3.4). The biotransformation of several sediment-carried PCBs could not be quantified in the spiked microcosms, as some of them were produced from the spiked congeners dechlorination. However, the fate of about 30% of the GC-ECD peaks ascribed to pre-existing PCBs could be monitored and compared with that observed in the non spiked microcosms (Table 3.2). A significant transformation of such pre-existing PCBs and of the exogenous PCBs was detected starting from the 5th month of incubation. However, the dechlorination of the exogenous PCBs only slightly influenced the bioconversion extent and pattern of pre-existing PCBs (Table 3.2), suggesting that the dechlorination of the latter was not significantly primed by the addition of the 5 exogenous coplanar congeners.

Table 3.2. Changes in the PCB concentration (µmoles/kg of dry sediment) in the biologically active non spiked and spiked microcosms (all estimated vs. the sterile ones) at the end of the 16th month of incubation.

Changes in PCB concentrations – active vs. sterile microcosms (µmoles/kg of dry sediment)		
PCB congeners	Non spiked microcosms	Spiked microcosms
2,6-2,2'-CB	-0,129 ± 0,072	-0,108 ± 0,062
2,4'-2,3-CB	-0,021 ± 0,020	0,034 ± 0,032
2,2',5-2,2',4-4,4'-CB	0,021 ± 0,015	-0,052 ± 0,009
2,2',3-2,4',6-CB	0,007 ± 0,002	-0,016 ± 0,002
2,2',3,6'-CB	-0,010 ± 0,006	-0,020 ± 0,001
2,2',3,4-2,3,4',6-CB	-0,018 ± 0,008	-0,037 ± 0,003
2,3,4',5-CB	-0,004 ± 0,007	-0,024 ± 0,002
2,3',4,4'-2,2',3,5',6-CB	-0,181 ± 0,069	-0,239 ± 0,041
2,2',3,4',6-CB	-0,003 ± 0,002	-0,006 ± 0,002
2,2',3',4,5-CB	-0,013 ± 0,007	-0,024 ± 0,001
2,2',3,4,5'-CB	-0,001 ± 0,005	-0,017 ± 0,004
2,2',3,4,4',5'-CB	-0,261 ± 0,138	-0,289 ± 0,144

The exogenous PCBs were markedly bioconverted into less chlorinated congeners, such as 3,3’,5,5’-/2,3’,4,4’-, 2,3’,4’,5- and 2,4,4’,5-tetrachlorobiphenyl, 2,4,4’-, 2,3’,4- and 2,3’,5-trichlorobiphenyl and 3,4- and 3,4’-dichlorobiphenyl between the 5th and the 8th month of incubation, and were found to be depleted by more than 80% at the end of the experiment (data not shown). This finding is of great relevance, as it indicates that indigenous microbial consortia selected in the spiked primary microcosms were able to rapidly and extensively dechlorinate some dioxin-like coplanar PCBs that are regarded as some of the most toxic PCBs reported in the literature[104]. The extensive dechlorination of the spiked PCBs only slightly intensified the rate and extent of the dechlorination of the PCBs pre-existing in the sediment. The lack of significant priming effects in aged PCB contaminated marine sediments has also been observed in a different contaminated sediment of the Venice Lagoon spiked with 2,3,4,5,6-pentachlorobiphenyl[102] and in a sediment of the LCP Chemicals Superfund site in coastal Georgia (USA)[97].

Taken together, these data suggest that PCB pre-existing in the contaminated sediment of Porto Marghera employed in this study can undergo microbial reductive dechlorination. This finding supports previous observations on the occurrence of dechlorination processes in contaminated sediments of the Venice Lagoon[96,102]. As all these laboratory studies were performed under geochemical conditions that mimick those occurring *in* *situ,* it is possible to speculate that such processes might be also in progress *in situ* in the lagoon of Venice.

Both sulfate-reduction and methane production occurred sequentially in the biologically active microcosms; PCB reductive dechlorination became detectable when sulfate was completely depleted and methanogenesis started to be significant. Thus, sulfate-reducing bacteria capable of dechlorinating PCBs in the absence of sulfate or methanogenic bacteria were probably responsible for the detected PCB biodegradation processes. The first hypothesis is supported by the work of Zwiernik et al.[105] and by the results of our recent work carried out on another contaminated sediment of Venice Lagoon[102], whereas the second one is consistent with the findings reported by Alder et al.[95] on the aged PCB-contaminated sediment of the New Bedford Harbor. However, it cannot be excluded that either sulfate reducing bacteria and methanogenic bacteria along with fermentative bacteria, that generally strictly interact with methanogenic and sulfate-reducing bacteria in anaerobic environments[106], played a direct role in the PCB dechlorination, as already proposed by other authors[94,107].

To gain deeper insights on the microorganisms potentially involved in the process, a culture enrichment program was started by developing new microcosm sets where the same sediment, spiked with the 5 coplanar PCBs, was progressively applied at lower percentages. Spiked PCB-dechlorination rate increased by about 70% by moving from the primary to the secondary microcosm (developed with sediment in site water at 25% v/v) and by over 100% to the tertiary microcosms (developed with 12% or 6% v/v of sediment). A remarkable increase in sulfate-reduction rates and a progressive decrease in the methanogenic activity were also detected during the serial transfers of the culture, suggesting that an enrichment of sulfate-reducing bacteria likely occurred and supporting their possible involvement in the dechlorination process. Molecular analyses of the microbial communities occurring in these enriched microcosms are currently in progress.

3.3. CONCLUSIONS

The occurrence of microbially-mediated, reductive dechlorination processes towards weathered PCBs and spiked high chlorinated and co-planar PCBs has been demonstrated in 2 contaminated sediment of the Brentelle Canal of the Porto Marghera area (Venice lagoon, Italy). The detected processes exhibited *meta*- and *para*-specificity (apparently they proceed through dechlorination pattern H’ and M) and were not significantly “primed” by the dechlorination of exogenous PCBs, that were rapidly and extensively dechlorinated. PCB dechlorination seemed to be mediated by sulfate-reducing bacteria, that probably started to use PCBs as electron acceptors during sulfate reduction but in particular when their native electron acceptor was completely depleted.

Such activities were detected under geochemical conditions that closely mimic those occurring *in situ*, and this allows to speculate that similar processes are also in progress *in situ*. However, the detected PCB dechlorination processes were slow and partial. In general, microbial processes occurring *in situ* are very constrained temporally and spatially. Further, natural sediment systems are complex, heterogeneous, and subjected to (bio)turbation phenomena and low and variable temperatures. Thus, the findings described above do not necessarily prove that biodegradation will provide sufficient natural *in situ* decontamination of the site but for sure, if properly combined with other lines of microbial and biogeochemical evidence coming from the same site[101], a strong indication of biodegradation *in situ*, and will justify further investigations.

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4. Sediments as a biobarrier for CAH-polluted groundwater

4.1. DEGRADATION OF CONTAMINANTS IN THE INTERFACE ZONE OF THE TRANSFER OF GROUNDWATER TO SURFACE WATER

Contaminated groundwater reaching surface waters such as rivers and lakes is considered to be an important source of continuous pollution of these surface water bodies. This is especially the case in industrial, urban and agricultural areas that are often located near surface water bodies. However, the fate of the infiltrating groundwater pollutants might be influenced by the sediment zone in eutrophic water bodies since such sediments possess characteristic biological and physico-chemical degradation properties[108-111]. Considering the generally high abundance of bacteria in sediments and the high bacterial activity therein, these microorganisms could play a major role in the degradation of groundwater pollutants infiltrating the sediment zone. Conant *et al.*[112] studied a tetrachloroethene (PCE) groundwater plume discharging into a river and showed that the near-river zone strongly modified the distribution, concentration and composition of the plume prior to discharging into the surface water. Of the various factors affecting the plume, the extensive anaerobic biodegradation that occurred in the shallow streambed deposits had the greatest effect because it altered the composition and potentially changed the overall toxicity of the plume[112].

Knowledge on natural attenuation of passing pollutants and the potential to stimulate and sustain occurring degradation processes in the sediment zone are however scarce. This is especially due to the lack of appropriate monitoring devices and tools to measure *in situ* mass balances of pollutants and other reactants/products.

4.2. STUDY OF THE SEDIMENTS OF THE RIVER ZENNE (BELGIUM) AS A BIOBARRIER AGAINST THE DISCHARGE OF CAH-CONTAMINATED GROUNDWATER INTO A EUTROPHIC RIVER

We evaluated the intrinsic capacity of eutrophic river sediment microbial communities to degrade Chlorinated Aliphatic Hydrocarbons (CAHs). The Belgian river Zenne at Vilvoorde was selected for this study. In this area, the Zenne drains groundwater polluted with vinyl chloride (VC) and *cis*-1,2-dichloroethene (*cis*-DCE). In a first instance, the concentrations of CAHs discharging into the Zenne were determined by the sampling of groundwater in monitoring wells and Geoprobe direct push sampling techniques (screenpoints) followed by a piston drill sampling of the interstitial water in the river bed sediment. In addition, anaerobic microcosms were constructed using sediment material obtained from different positions in the interface of the CAH-polluted part of the river Zenne. These microcosms, and molecular biological techniques were used to study the CAH-degradation potential of the microbial population of the eutrophic river sediments. The information obtained will indicate if the sediments can be used as a natural biobarrier against the infiltration of the CAH pollution into the surface water.

4.2.1. *Determination of the CAH-influx zones in the zenne riverbed*

In Vilvoorde (Belgium), a 1,2 km-wide CAH groundwater plume, originating from former industrial activities, extends downgradient of multiple source zones to the river Zenne. Previous research indicated that PCE, that was spilled at the sources, is successively converted to TCE, DCE and mainly VC in the aquifer while the groundwater is flowing towards the Zenne.

To determine the zones where the highest CAH concentrations are reaching the river, a screen point measurement campaign was organized. During this campaign, temporary monitoring wells (screen points) were installed along the side of the Zenne at a distance of approximately 5 m from the river and 125 m from each other. Water samples were taken at a depth of 7 and 10 m-bgs at these screen points and in selected monitoring wells. The obtained water samples were analyzed, determining the concentration of CAH, ethene and ethane in the laboratory by, respectively, GC-MS and GC-FID analyses.

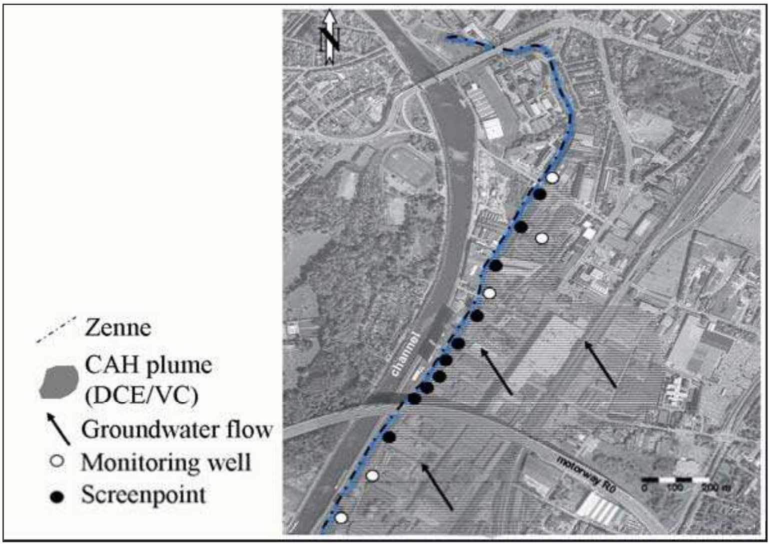

Figure 4.1. Site map showing CAH groundwater plume that is discharging into the river Zenne.

Figure 4.1 represents the positions of the screen points and monitoring wells along the Zenne that were sampled. Fig. 4.2 indicates the measured concentration of CAH and ethene in the obtained water samples.

The results indicate that at a depth of 7 m-bgs, mainly VC and to a lesser extent DCE are flowing into the Zenne (Fig. 4.2). The highest concentrations of VC were measured in screen point n°4 (154 µg/L), screen point n°5 (677 µg/L) and 6 (127 µg/L). Samples obtained at a depth of 10 m-bgs contained next to VC also a high concentration of DCE (Fig. 4.2). These compounds were again mainly found in screen points n°5 (VC: 2212 µg/L; DCE: 150 µg/L) and 6 (VC: 743 µg/L; DCE: 0 µg/L). From these results we concluded that the VC-plume is mainly flowing into the Zenne in the area between screen point n°5 and 6. To narrow the area of investigation, three new screen points were drilled (screen points 5.1, 5.2 and 5.3, approximately 40 m apart and located between screenpoints n°5 and 6). Based on the analysis results we selected a 50 m long test area in the river Zenne (containing screenpoints 5 and 5.1. See rectangle on Fig. 4.2) at which all further measurements and sampling were conducted.

Next to VC and DCE, also the degradation products ethene (Fig. 4.2) and ethane (data not shown) were detected. This indicates that complete degradation of the groundwater pollutants VC and *cis*-DCE to their non-toxic end products ethene and ethane is occurring. However, at some locations, this microbial reductive dechlorination rate clearly does not suffice to prevent the groundwater pollutants from infiltrating into the river.

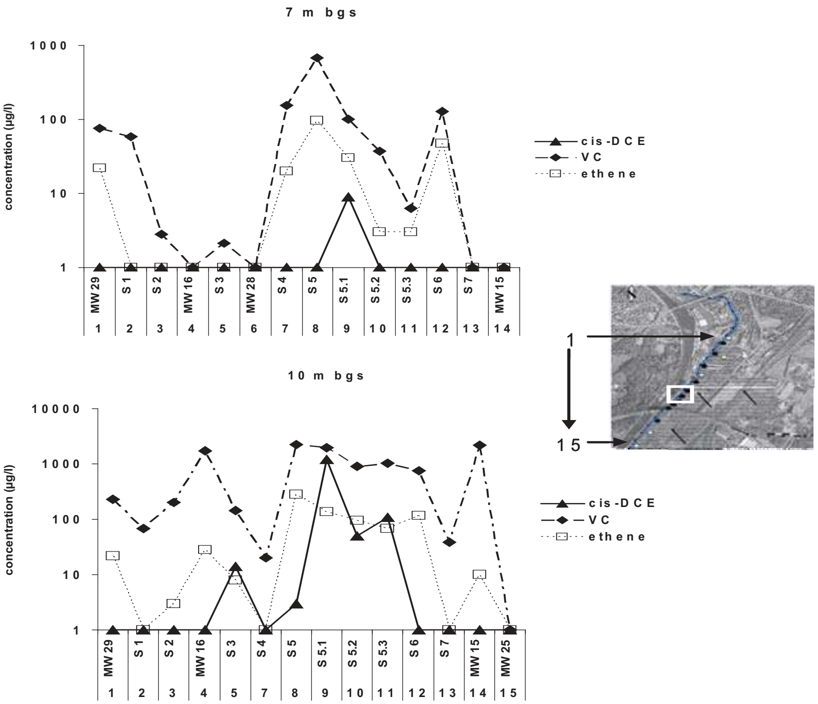

Figure 4.2. Representation of the measured VC, *cis*-DCE and ethene concentrations in the water sampled in the screen points (S) and monitoring wells (MW) in the Zenne area. The rectangle indicates the selected 50 m long test field.

To determine the locations where groundwater discharges into the surface water and the respective CAH concentrations in the interstitial water at these locations, a piston drill sampling of the riverbed sediment was performed in the selected test area, stretching from ‘mooring post 26’ up to a location 50 m upstream of this post (Fig. 4.3). Every 5 m a sample was taken over a total length of 50 m and this at the right, middle and left side of the Zenne (Fig. 4.3). For the obtained sediment cores, the structure of the river core was described and sub-samples were taken at depths of appr. 10, 50 and 100 cm in the core to determine the concentration of CAHs (by GC-MS analysis), ethene, and ethane (by GC-FID analysis), pH and redox potential.

Apparently, the concentrations of the CAHs that are flowing into the Zenne depend on the sampling place in the river. The spatial distribution of the concentration of VC is shown in Fig. 4.4. At post 26, no VC is flowing into the river. Analysis of the texture of the samples that were taken in this

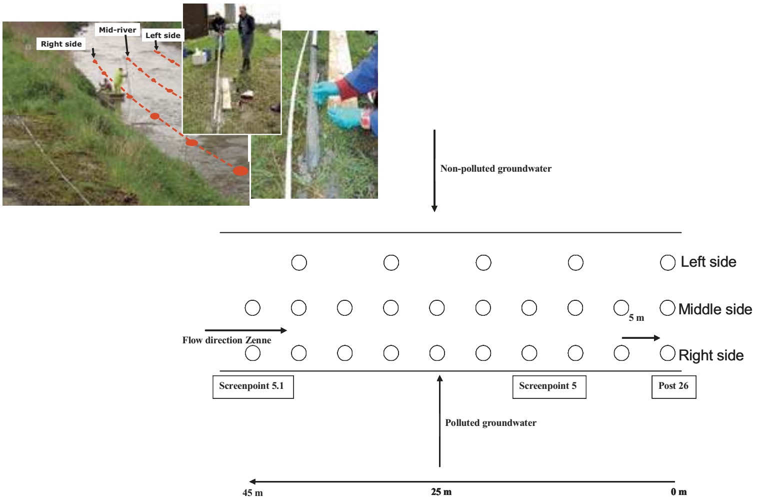

Figure 4.3. Piston drill sampling at multiple locations in the streambed of the river Zenne.

last area indicated that they contain layers with very fine sand that could prevent the infiltration of VC into the river. In the area from 15 m to 45 m upstream of post 26, VC is flowing into the Zenne and the highest concentrations are detected between 25 and 30 m upstream of this post (> 1000 µg/L). While VC is detected throughout the studied Zenne area, *cis*-DCE is only present between 30 m and 40 m upstream of post 26 at a concentration of 27 to 300 µg/L (data not shown).

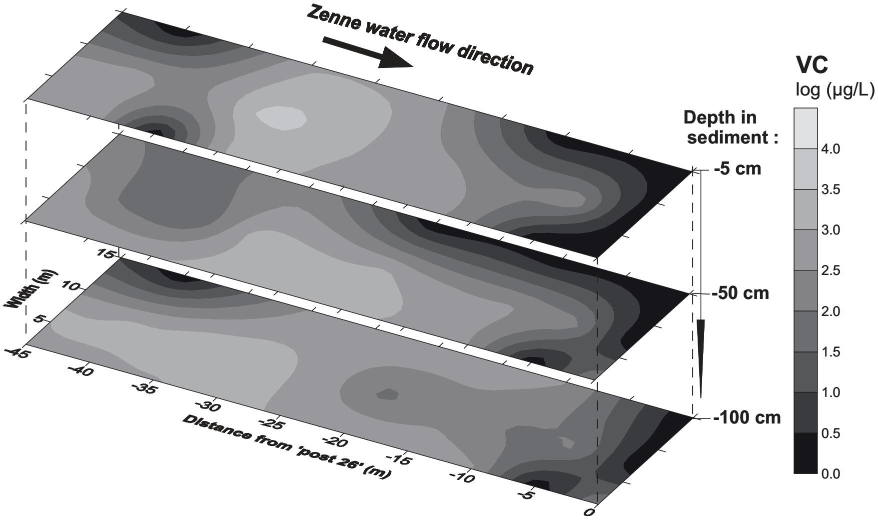

Figure 4.4. Measured interpolated VC and *cis*-DCE concentrations in the pore water of the sampled sediment in the river Zenne at the three sampling lines (right, middle and left) and depths (10, 50 and 100 cm).

Depending on the position in the Zenne, the VC-concentration at the top of the piston drill samples (approximately 10 cm beneath the surface water) is lower, equal or higher than in the middle and the bottom of the cores (Fig. 4.4). However, at one location a VC concentration of 1139 µg/kg d.m. is detected in the top of the sediment layer. This indicates that VC is discharging into the surface water of the river Zenne. At other locations, e.g. closer to post 26, much lower VC concentrations are present. The concentrations of ethene and ethane that are detected (data not shown) indicate that full dechlorination of VC to ethene and ethane is occurring, but apparently not efficiently enough to prevent VC from flowing into the river.

It can be concluded that the groundwater discharge spatial distribution is highly heterogeneous, mostly depending on local sediment permeability. At some locations (e.g. at post 26) full dechlorination was observed, while VC and *cis*-DCE reach the surface water and discharge into the river at spots with a high-velocity groundwater influx.

4.2.2. *Determination of the degradation potential of the eutrophic river sediment microbial community by molecular analyses and anaerobic degradation tests*

To determine the degradation potential of the eutrophic river sediment microbial community, anaerobic microcosms and molecular biological techniques were applied on sediment material obtained from different positions in the interface of the CAH-polluted test area of the river Zenne.

The presence of dehalogenating bacteria and chloroethene dehalogenase genes was investigated in surface water, groundwater and sediment slices obtained from macro-core sediment samples (Fig. 4.5) collected at the right, middle and left side of the Zenne located around ‘mooring post 26’. The undisturbed sediment cores were frozen on dry ice immediately after sampling and divided into slices of approximately 0,5 cm in the laboratory, using an electrical saw. The presence of CAH degrading bacteria (*Dehalococcoides* and *Desulfuromonas* species) and genes involved in the first steps (*pce*A and *tce*A) and the last step (*tce*A, *vcr*A and *bvc*A) of the reductive dehalogenation of PCE to ethene was investigated by PCR[113-116].

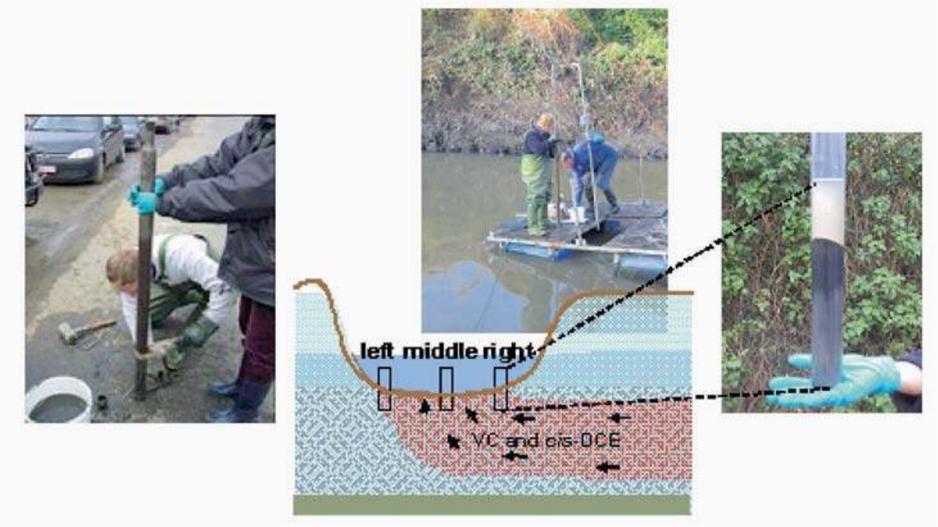

Figure 4.5. Macro-core sampling at three different places in the river bed and the obtained undisturbed sediment sample.

Only the PCR results of a sediment core obtained at the right side of the river are shown (Fig. 4.6). *Dehalococcoides* species, capable of degrading PCE completely to ethene, were present in the investigated groundwater and in all the sediment slices of the cores obtained from the right, middle and left side of the Zenne riverbed, but not in the surface water. *Desulfuromonas* species, capable of degrading PCE to *cis*-DCE, were also found in almost all sediment slices of the different cores and in the surface water of the river Zenne, but not in the groundwater. The *pce*A$^{(1)}$ gene of *Dehalobacter restrictus*, *Desulfitobacterium hafniense*, and *Desulfitobacterium* sp. PCE1, and the *pce*A$^{(2)}$ gene of *Sulfurospirillum multivorans* were only detected in some of the sediment slices throughout the river bed. The *pce*A$^{(2)}$ gene was also detected in surface water of the river Zenne. The *tce*A gene of *Dehalococcoides* sp. strain FL-2 and *D. ethenogenes* strain 195 was present in the groundwater, surface water and some or all sediment slices from the cores collected at the three different sampling places. The encoded TCE reductive dehalogenase can transform TCE to non-toxic ethene, but the reduction of VC to ethene is co-metabolic. The VC reductive dehalogenase genes of *Dehalococcoides* sp. strain VS and BAV1, *vcr*A and *bvc*A, were only detected in the sediment core collected from the right side and not in the middle or the left side of the Zenne. Since these two *Dehalococcoides* species can derive energy for growth from the degradation of *cis*-DCE and VC to non-toxic ethene as opposed to *D. ethenogenes* strain 195 and FL-2, their presence indicates that a large microbial degradation potential is present in the river sediment where polluted groundwater is passing the sediment zone.

The actual anaerobic degradation of VC and *cis*-DCE by the eutrophic river sediment microbial community was studied in anaerobic microcosms constructed with fresh Zenne sediment obtained from the top (5-10 cm) or the bottom (10-20 cm) of undisturbed sediment cores that were taken in the test area. A mixture of sediment material (37,5 g) obtained from the left and right side of the Zenne was suspended in 70 ml of groundwater that was collected directly upstream from the river using a Geoprobe direct-push system. The degradation of VC and *cis*-DCE was followed in function of time by headspace GC-FID analysis. The results of these batch degradation tests (Fig. 4.7 representing results 10-20 cm depth sediment material) indicate that both the *in situ* concentrations of VC (around 300 µg/L) and *cis*-DCE (around 150 µg/L) were rapidly reduced to non-toxic ethene and ethane both in the top and the bottom river sediment. When 2000 µg/L *cis*-DCE was re-added to the microcosms, it was again completely reduced to ethene within 1 month.

		Dehalococcoides	***Desulfuromonas***	***pce* A$^{(1)}$**	***pce* A$^{(2)}$**	***tce* A**	***vcr* AB**	***bvc* A**
surface water		▨	▨		▨	▨		
black	**20**	▨	▨		▨			▨
	19	▨	▨				▨	▨
	18	▨	▨		▨		▨	▨
	17	▨	▨				▨	▨
	16	▨	▨			▨	▨	▨
	15	▨	▨			▨	▨	▨
	14	▨	▨			▨	▨	▨
	13	▨	▨	▨		▨	▨	▨
	12	▨	▨	▨		▨	▨	▨
	11	▨	▨			▨	▨	▨
	10	▨	▨		▨	▨	▨	▨
gray	**9**	▨	▨				▨	▨
	8	▨	▨					▨
	7	▨	▨			▨		▨
	6	▨	▨					▨
	5	▨						▨
	4	▨				▨		▨
	3	▨	▨			▨		▨
	2	▨	▨			▨		
	1	▨	▨			▨	▨	▨
groundwater								
7m bgs		▨				▨		
10m bgs		▨				▨		

Figure 4.6. PCR results of the detection of dehalogenating bacteria and chloroethene dehalogenase genes in a sediment core obtained at the right side of the river Zenne. Boxes represent surface water, groundwater or the sediment slices that were obtained from the frozen sediment core. Empty boxes indicate that no PCR product was obtained, shaded boxes indicate the presence of the bacterium or catabolic gene.

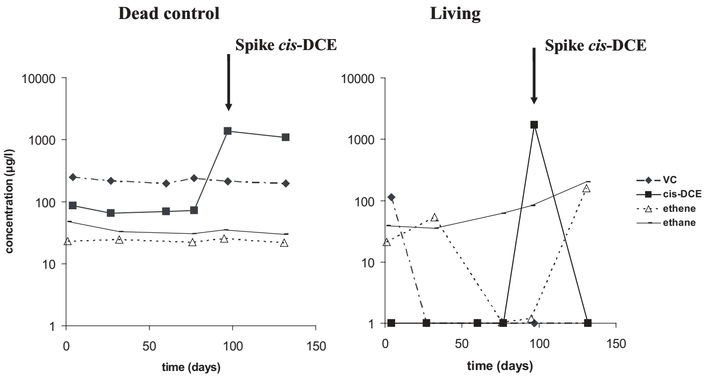

Figure 4.7. Anaerobic degradation of VC and *cis*-DCE in batch degradation tests containing (10-20 cm depth) river sediment material and groundwater from the CAH-polluted test area of the river Zenne.

4.3. CONCLUSIONS

A high microbial CAH degradation potential was detected in the river sediment of the Zenne in Belgium. The detected *Dehalococcoides* sp. strain VS and BAV1, which can actually grow on VC and *cis*-DCE as sole energy source, are probably responsible for the relatively fast and complete reductive dehalogenation of these groundwater pollutants that is observed in the microcosms. By degrading VC and *cis*-DCE, the sediments act as a natural biobarrier for the CAHs present in the groundwater that is passing through the sediment zone, hereby reducing the risk of surface water contamination.

However, some locations in the studied test area have high groundwater influx rates. At these locations VC and *cis*-DCE most likely reach the surface water and discharge into the river. At these locations, the observed microbial degradation potential of the river sediment apparently is inadequate to prevent the CAHs from reaching the surface water. Future research will therefore focus on how to stimulate the degradation of the CAHs, both in the river sediment and in the aquifer material upstream of the river Zenne.

5. Ex-situ and in-situ biotreatment of contaminated sediments

5.1. INTRODUCTION

Pollution of soils, sediments and groundwaters is a major problem worldwide. The total world hazardous waste remediation market is estimated at about US $16 billion per year. There are at least 350 000 contaminated sites in Western Europe alone and it may cost as much as US $ 400 billion to clean the most dangerous of these sites over the next 20-25 years[117]. Sediments provide essential habitat for many freshwater, estuarine, and marine organisms. In aquatic systems, most anthropogenic chemicals and waste materials, particularly persistent organic and inorganic chemicals, may accumulate in sediments. These sediments become repositories for many of the more toxic chemicals that are introduced into surface waters. These pollutants can attach to suspended particulates in the water, and subsequently settle out to the bottom. Through complex chemical, physical and biological interactions, these contaminants may be further transformed and transported to other parts of the aquatic system. At elevated concentrations, contaminated sediments lead to many problems in lakes, rivers and harbors, including fish advisories, habitat impairments and restrictions on dredging. Contaminated sediments may also pose an unacceptable risk to aquatic organisms, aquatic-dependent wildlife and humans. Contaminants that build up in the food chain are of particular concern, especially mercury, polychlorinated biphenyls (PCBs), dioxins and organochlorine pesticides. It has become clear that sediment cleanup is an important task and several remediation strategies are designed and implemented to eliminate toxic pollutants from these matrices. Physical and chemical treatments have been used to clean up sediments but they are usually very costly and they are not applicable to all types of environments. For instance, air or steam stripping is practiced primarily for the cleanup of unsaturated soils, whereas “pump and treat” technologies target mainly the groundwater (and sometimes saturated soil and sediment zones), involving pumping and capturing of contaminants (e.g. on a sorbent or by skimming, flotation, etc.) from the water, which is returned to the aquifer. This physical flushing of the pollutants from large volumes of groundwater or saturated sediment is extremely slow and costly. Washing of the solid matrix is also practiced, but is mainly of use on unsaturated soils and less so on saturated soils and sediments. Finally, incineration is a drastic measure, addressing soils and sediments that are contaminated with weathered, often intimately encrusted recalcitrant pollutants. Its cost is high and the environmental impacts can be severe.

Bioremediation strategies are less expensive and friendlier to the environment than physical and chemical treatments of contaminated soils and, to a lesser extent, sediments. This set of technologies uses biological, primarily microbial, activities and can be classified into three major techniques:

1. *In situ*: No excavation, the solids remain in place. This class of techniques can be subdivided into engineered in situ bioremediation and intrinsic bioremediation (or natural attenuation). Examples include bioventing, biosparging, etc.
2. *Ex situ*: Sediment is excavated and transferred to be treated in specialized installations far from the site. It is always an engineered solution. Examples: land farming, slurry reactors. A sub-category of ex situ bioremediation, occasionally considered as a separate class of techniques, is *on site* bioremediation, which implies treatment of a soil or sediment at the surface of the site (e.g., biopiling).

The applicability of different biological treatments depends on the susceptibility of the pollutants to biodegradation (Table 5.1) and on the type of environment targeted for cleanup. A number of prior steps are needed before deciding on the bioremediation strategy, including a thorough characterization of the site in terms of hydrology, extent and type of contamination, intrinsic microbial activity, and supply rates of key materials, such as electron acceptors or donors[118].

Table 5.1. Contaminant susceptibility to bioremediation.

Pollutant	Frequency of occurrence	Status of bio-remediation	Limitations
Polycyclic aromatic hydrocarbons	Common	Emerging	Sorb strongly to subsurface Solids
Chlorinated aliphatics	Very frequent	Emerging	Nonaqueous phase liquids (NAPL)
Chlorinated aromatics	Common	Emerging	NAPL and solids
Polychlorinated biphenyls	Infrequent	Emerging	Sorb strongly to subsurface Solids
Heavy metals & metalloids	Common	Possible to immobilize	Availability highly variable
Gasoline, fuel oil	Very frequent	Established	NAPL

5.2. *IN SITU* BIOREMEDIATION

In situ bioremediation encourages growth and reproduction of indigenous microorganisms to enhance biodegradation of organic constituents in the saturated zone. *In situ* groundwater bioremediation can effectively degrade organic constituents which are dissolved and adsorbed onto the aquifer matrix. In an analogous manner, a sediment contaminated with organic pollutants can be cleaned up. *In situ* bioremediation can be effective for the range of xenobiotic compounds indicated in Table 5.2. In certain cases *in situ* bioremediation is more effective when combined with other saturated zone remedial technologies (*e.g.*, air sparging) and, for the case of contaminated soils, with vadose zone remedial operations (*e.g.*, soil vapor extraction, bioventing). Air sparging involves the injection of compressed air or oxygen directly into the contaminated subsurface. Injected air traverses horizontally and vertically in channels through the sediment column, creating an underground stripper that removes volatile and semivolatile organic contaminants by volatilization and entrainment in the gas stream. The injected air helps to flush the contaminants into the unsaturated zone. Soil Vapor Extraction (SVE) usually is implemented in conjunction with air sparging to remove the generated vapor-phase contamination from the vadose zone. Oxygen added to the contaminated groundwater and vadose-zone soils also can enhance biodegradation of contaminants below and above the water table by stimulating the local microbial activity. Bioventing is an *in situ* remediation technology that combines soil vapor extraction methods with bioremediation. It uses vapor extraction wells that induce air flow in the subsurface through the use of a vacuum. Bioventing can be effective in remediating emissions of petroleum products, such as gasoline, jet fuels, kerosene, and diesel fuel but is mostly applicable to unsaturated matrices. Since the contaminated sediment represents a partly or totally saturated zone, a water circulation strategy for the supply of metabolism stimulating materials can often be applied *in situ,* e.g. for the engineered bioremediation of a nonaqueous phase liquid such as petroleum. In this case, hydrogen peroxide can be used as a dissolved source of oxygen, via vertical wells and horizontal infiltration galleries[118,119] (see below).

5.2.1. *Biostimulation and bioaugmentation*

In situ bioremediation can be implemented in a number of treatment modes, including: Aerobic (oxygen respiration); anoxic (nitrate respiration); anaerobic (non-oxygen respiration); and co-metabolic. The aerobic mode has been proven most effective in reducing contaminant levels of aliphatic (*e.g.*, hexane) and aromatic petroleum hydrocarbons (*e.g.*, benzene, naphthalene) typically present in gasoline and diesel fuel. In the aerobic treatment mode, the sediment and aquifer area is oxygenated by one of three methods: Direct sparging of air or oxygen through an injection well; saturation of water with air or oxygen prior to re-injection; or addition of hydrogen peroxide directly into an injection well or into reinjected water. Irrespective of the method of oxygenation, it is important to ensure that oxygen is being distributed throughout the area of contamination. Anoxic, anaerobic, and co-metabolic modes are sometimes used for remediation of other compounds, such as chlorinated solvents, but are generally slower than aerobic respiration in breaking down xenobiotics. The key parameters that determine the effectiveness of *in situ* bioremediation are: (a) hydraulic conductivity of the aquifer, which controls the distribution of electron acceptors and nutrients in the subsurface; (b) biodegradability of the xenobiotic constituents, which determines both the rate and degree to which constituents will be degraded by microorganisms; and (c) location of the xenobiotic contamination in the subsurface. In general, the sediment/aquifer system will determine hydraulic conductivity. Fine-grained media (*e.g.*, clays, silts) have lower intrinsic permeability than coarse-grained media (*e.g.*, sands, gravels). Bioremediation is generally effective in permeable media. However, depending on the extent of contamination, bioremediation also can be effective in less permeable silty or clayey media, which represent a considerable proportion of sediments. In general, a medium of lower permeability will require a longer period of time to clean up than a more permeable medium[3]. The biodegradability of a xenobiotic constituent is a measure of its ability to be metabolized (or co-metabolized) by microorganisms. The chemical characteristics of the contaminants will dictate their biodegradability. For example, heavy metals are not degraded by bioremediation but they can be sequestered as insoluble precipitates under specific redox conditions. The biodegradability of organic constituents depends on their chemical structures and physical/chemical properties (*e.g.*, water solubility, water/octanol partition coefficient). Highly soluble organic compounds with low molecular weights will tend to be more rapidly degraded than slightly soluble compounds with high molecular weights. The low water solubilities of the more complex compounds render them less bioavailable to microorganisms. Consequently, the larger, more complex chemical compounds may be slow to degrade or may even be recalcitrant to biological degradation[118,119].

When oxygen is introduced to the subsurface as a terminal electron acceptor, it can react with dissolved iron [Fe(II)] to form an insoluble iron precipitate, ferric oxide. This precipitate can be deposited in infiltration trenches or channels, reducing permeability. The effects of iron precipitation tend to be most noticeable around injection wells, where oxygen concentration in groundwater is highest and can render injection wells inoperable.

Extreme pH values (*i.e.*, less than 5 or greater than 10) are generally unfavorable for microbial activity. Typically, optimal microbial activity occurs under neutral pH conditions (*i.e.*, in the range of 6-8). The optimal pH is site specific. For example, aggressive microbial activity has been observed at lower pH conditions outside of this range (*e.g.*, 4.5 to 5) in natural systems. Because indigenous microorganisms have adapted to the natural conditions where they are found, pH adjustment, even toward neutral, can inhibit microbial activity. If man-made conditions (*e.g.*, releases of petroleum) have altered the pH outside the neutral range, pH adjustment may be needed. If the pH of the groundwater is too acidic, lime or sodium hydroxide can be added to increase the pH. If the pH is too alkaline, then a suitable acid (*e.g.*, hydrochloric) can be added to reduce the pH. Changes to pH should be closely monitored because rapid changes of more than 1 or 2 units can inhibit microbial activity and may require an extended acclimation period before the microbes resume their activity.

As a rule, microorganisms require carbon/energy sources (electron donors) and terminal electron acceptors (TEA) to enzymatically transform the target contaminants. Carbon source(s) and TEA are introduced in *in situ* engineered bioremediation systems via injection wells or infiltration galleries. Extraction wells ensure that the pollutant plume does not spread contaminants into clean areas or accelerate the movement toward receptors. Placement of extraction wells is critical, especially in systems that also use nutrient injection wells or infiltration galleries. These additional sources of water can alter the natural groundwater flow patterns which can cause the contaminant plume to move in an unintended direction or rate. Without adequate hydraulic control, this situation can lead to worsening of the original condition and complicate the cleanup or extend it.

Nutrient injection systems may be unnecessary if the sediment/ groundwater system contains adequate amounts of nutrients, such as N and P, to sustain microorganism growth and biodegradation. Nutrients may be available in sufficient quantities in the sediment/aquifer system (intrinsic *in situ* bioremediation, in which no external intervention is taking place other than a careful monitoring of the natural attenuation of the target contaminants) but, more frequently, nutrients need to be added to maintain adequate bacterial populations (biostimulation)[118,119]. Addition of N, P and other inorganic nutrients to optimize microbial growth is becoming established for in situ bioremediation of subsurface contaminated by hydrocarbons. Supply of electron donors (e.g. sugar or methane) or TEA (e.g. oxygen or hydrogen peroxide) in case of such limitations, or addition of detergents in case of low bioavailability of the pollutants, are instances of biostimulation[119].

Sometimes the microbial community of a polluted site may not display the metabolic potential for degradation and complete mineralization of the target pollutant. This could be due to very low numbers of a single microorganism possessing the entire pathway for its eventual breakdown to harmless end products. More likely, the pollutant may be a complex molecule or a mixture of compounds which could be broken down only by a specific combination of microorganisms ('consortium') and pathways. In such cases, successful bioremediation may call for bioaugmentation, i.e. the inoculation of the polluted sediment with specific populations of microorganisms[120]. Bioaugmentation is not yet a mature technology for in situ bioremediation of soils and groundwater and, even less so, of sediments. With the exception of a few field applications most bioaugmentation reports concern laboratory-scale studies. It is then no surprise that bioaugmentation is mired in controversy, given the excessive claims of success, esp. by commercial companies, and the documented failures of large-scale inoculation campaigns[119,120]. Typical bioaugmentation options include: the addition of a pre-adapted pure bacterial strain (e.g. inoculating with chloroaromatic degraders); the addition of pre-adapted consortia (e.g. PCB-degrading enrichment cultures to contaminated sediments); addition of genetically modified bacteria for simultaneous mineralization of chloro- and methylaromatics; and transfer of biodegradation-relevant genes by conjugation into microorganisms present in the biotope to help remove PCBs or pesticides[120]. Ideally, the applicability and the limits of bioaugmentation should be tested in a situation comparable to the eventual intervention, i.e., at a mesocosm or pilot scale, after initial microcosm tests. In the first study of bioaugmentation of a realistic 500-liter pilot-scale reactor simulating an anaerobic aquifer/sediment system, we tested the capacity of an inoculum of the halorespiring anaerobe *Desulfomonile tiedjei* to dechlorinate 3-chlorobenzoate (3-CB) continuously fed into the saturated zone together with acetate+formate as cosubstrate[121]. Heterogeneous distribution of 3-CB dechlorination activity correlated with the presence of the inoculated bacterium which was detected by its 16S rRNA gene signature using PCR. Denaturing gradient gel electrophoresis (DGGE) of PCR amplicons from the total DNA of different compartments in the reactor demonstrated distinct community fingerprints as a function of location and in response to process modifications[121]Reductive dehalogenation of organic pollutants in groundwater following biostimulation of native microbial communities is often seen in the field, where chlorinated ethenes (PCE, TCE) are completely transformed to ethene[122]. According to industrial practitioners of *in situ* bioremediation in the field, bioaugmentation with specific halorespiring anaerobes of the genus *Dehalococcoides*, which degrades chloroethenes to ethene without stopping at *cis*-dichloroethene (cDCE) or at vinyl chloride (VC), may shorten the lag phase close to the injection wells, but is not required to assure complete *in situ* dechlorination. Moreover, because it is not necessary to constrain hydrogen levels for the benefit of inoculated *Dehalococcoides* sp., it is also not necessary to limit rates of electron donor (cosubstrate) consumption by using commercial "designer" slow-release cosubstrates[122]. In a pilot investigation consistent with this standpoint, we examined on a demonstration (pilot) scale the dynamics of *in situ* bioremediation of a sediment co-contaminated with trichloroethene (TCE) and nickel[123,124] in preparation for the field scale *in situ* intervention at the former metallurgical industrial site of Bunnik near Utrecht (The Netherlands), where the contaminated anaerobic sediment was located 20-30 m below ground. The 680-liter "sandbox-type" reactor filled with the contaminated sediment was fed under strict anaerobic conditions a 10 mg/l TCE-containing stream simulating the plume together with a methanol+lactate cosubstrate mixture. The cosubstrate chemical oxygen demand (COD) was progressively diminished from 1000 mg/l down to 100 mg/l as the redox potential reached levels consistent with sulfate reduction and methanogenesis while the feed COD/$SO_4^{-\,-}$ ratio was fixed at 100/0.6 (w/w)[123]. In previous small-scale fed-batch sediment column tests, this cosubstrate mixture had been shown to be a good electron donor to sustain complete TCE dechlorination, and a level of sulfate up to 10 mM not only did not interfere with the complete transformation of TCE to ethane, but also contributed to Ni sequestration in the form of NiS[124]. Results of the pilot reactor showed that TCE was dechlorinated to harmless ethene via cDCE and VC by indigenous microorganisms in the sediment, whereas concurrent stimulation of sulfate-reducing anaerobic bacteria with the fed sulfate led to the generation of sulphide which was efficient in precipitating nickel within the sediment[123]. Remarkably, probing with 16s rDNA –based PCR gave no indication of the presence of dechlorinating bacteria of the *Dehalococcoides* group, which indicates that the complete dechlorination was brought about either by as yet unknown halorespirers or cometabolically by local anaerobic trophic groups including sulphate reducers[124]. The dynamics of the process is shown in Fig. 5.1.

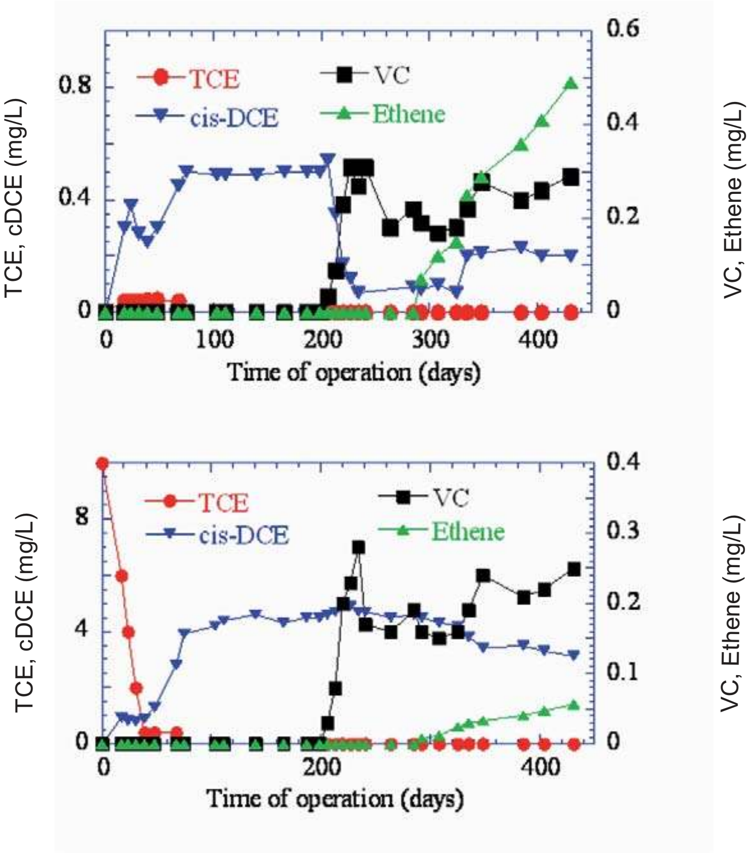

Figure 5.1. Time course of the concentrations of pollutant and products in the demonstration-scale sediment bioreactor. Chloroethene concentrations at the reactor outlet in the gas phase (upper panel) and in the liquid phase (lower panel).

5.3. *EX SITU* BIOREMEDIATION

Above ground treatment of contaminated sediments is an option in the case of limited of quantities of heavily contaminated sediments, and whenever regulatory compliance or other constraints impose a rapid cleanup[118,119]. This set of technologies, which includes slurry bioreactor treatment, land farming and composting, should only be practiced when the usually high costs of excavation and exposure to workers and residents can be more than compensated by a major risk reduction stemming from the removal of the pollutant source.

Slurry bioreactor treatment involves vigorous agitation of the sediment which may constitute 5-20% of the solids in a two- or three-phase configuration (depending on whether there is aeration or not) in the presence of native or, occasionally, externally introduced competent microorganisms. The degree of control and optimization that can be exercised on this system is considerable: in addition to vigorous inocula, nutrients, TEA and other additives can be introduced as needed. Therefore, the endpoints of this treatment are also far more predictable than in *in situ* bioremediation. Because of the high degree of convective mass transfer in this setup, the rates of biotransformation and mineralization of the target contaminants are several-fold higher than what is observed *in situ*. In addition, mass transfer into the aqueous phase and, hence, overall rates of biodegradation in the case of less bioavailable, poorly water soluble compounds (such as PCB, PAH etc.), can be enhanced with the addition of solubilizing agents such as surfactants[117,118].

Land farming involves the mixing of contaminated sediment into the surface layer of topsoil, to which there is an occasional addition of nutrients and moisture, the aim being an aerobic cometabolic degradation of the target pollutants by the soil microorganisms[118]. This empirical, generally uncontrolled, “low” technology can be upgraded by mixing the contaminated solids with fresh organic residues, resulting in composting[117]. The addition of air by appropriate channeling or turning as well as the regulation of moisture (and nutrient or cosubstrate input) can enhance the rates of pollutant degradation and also increase the overall degree of control and predictability of the treatment of sediments. Nonetheless, both these techniques can be cumbersome, time-consuming and expensive: in addition to the costs of excavation, transportation and mechanical intervention (e.g. turning, tilling, etc.), they do require ample land areas. Moreover, certain very recalcitrant contaminants, even if immobilized or rendered non-bioavailable by sorption and/or covalent binding to the humic acids of the compost or to other components of the soil matrix may present a risk of eventually leaching out under extreme local conditions.

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UNCERTAINTY AND RESEARCH NEEDS IN THE AREA OF THE BIOLOGICAL RESTORATION OF CONTAMINATED SEDIMENTS

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1. Background: Microbial processes in sediments and their potential role in the contaminated sediments remediation

A large array of microbial processes are taking place in aerobic or subsurface anaerobic sediments, where they are responsible for the turnover of naturally occurring organic matter and the N, P, S geochemical cycles (Kafkewitz and Togna, 1998). Oxidizing conditions generally exist only at the top few centimeters of surface sediments, providing a limited zone where oxygen is available as electron acceptor for aerobic biotransformation of organic and inorganic chemical compounds. Beneath the aerobic zone, sediments are typically dominated by a single electron acceptor process such as sulfate-reduction in marine sediments or methanogenesis in freshwater sediments. Bacteria and eukaryotic organisms are coexisting in such matrices, by strictly interacting and cooperating through complex and often not fully elucidated mechanisms. The microbial population might be responsible for the biodegradation/biotransformation of several chlorinated priority pollutants, such polychlorinated-biphenyls, -dibenzodioxins, -dibenzofurans, -phenols and -benzenes, as well as some hydrocarbons, in particular under anaerobic conditions. Under sulfidogenic conditions, it might be also responsible for the precipitation/immobilization of some toxic heavy metals (Lloyd and Lovley, 2001). These microbial processes might be in turn responsible for a significant and cost effective decontamination/detoxification of polluted sediments (Bedard and Quensen, 1995; Lloyd and Lovley, 2001; Haggblom et al., 2003). This potential becomes of special relevance when they are actively taking place

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in situ, where they might contribute to a significant mitigation of contamination (Natural Attenuation, NA) (Apitz et al., 2004). NA often results in a significant reduction of the area (volume of contaminated sediment) to be dredged or managed through suitable *in situ* physical-chemical treatments.

However, many among such observations are based on preliminary and/or incomplete/inadequate experimental evidence (Bedard and Quensen, 1995; Lloyd and Lovley, 2001). In fact, relatively little is known yet about the actual relevance of microbially mediated degradation/detoxification processes, such as those mentioned above, *in situ*, and in particular in the large number of marine contaminated habitats (Bedard and Quensen, 1995; Haggblom et al., 2003), often impacted by marked advective processes (Apitz et al., 2004). The few data coming from *in situ* monitoring (Monitored Natural Attenuation, MNA) generally indicate that microbiologically mediated biodegradation processes are slow, partial and very often constrained spatially and/or temporally (Apitz et al., 2004). Almost nothing is currently known about possible strategies/approaches suitable to efficiently and safely stimulate such processes *in situ*. These and other gaps of information on several other basic issues related to biological removal of pollutants from sediments (issues that are listed and discussed below) and on their actual relevance *in situ*, have dramatically reduced and are still adversely affecting the opportunities and perspectives of biological approaches in the management of the huge amounts of contaminated sediments.

2. Uncertainties in the area of biological treatment of contaminated sediments

2.1. UNCERTAINTIES ON BASIC ASPECTS OF MICROBIAL TRANSFORMATION OF POLLUTANTS IN SEDIMENTS

The biotransformation potential of microorganisms occurring in contaminated sediments is largely controlled by the pollutant bioavailability (Bedard and Quensen, 1995; Alexander, 1999; Haggblom et al., 2003). The value of this parameter is generally very low in sediments, in particular for highly hydrophobic pollutants, because of the generally very high content of sorbing or otherwise sequestering materials within the matrices. Microbes are also important determinants for this parameter, as they significantly contribute to the sediment redox potential, pH, precipitation and dissolution of minerals that compose the surface of sediments. Microbial degradation processes are probably able to remove the pollutants *per se* (assuming a relative abundance of competent microorganisms) and the fraction of each pollutant loosely bound to the matrix (Alexander, 1999; Apitz et al., 2004).

Thus, a first point of uncertainty is whether we have to spend time and financial resources aiming at enhancing/speeding up the release of tightly bound contaminants out of sediments and if we can ever have an effective and biocompatible strategy to do so. Another uncertainty is related to the actual toxicity of the non-bioavailable pollutant fraction that, due to this feature, is remaining in the sediments. A further discussion of bioavailability and of its possible implications on sediment toxicity and biological restoration is available at the following web-site: http://www.sediments.org./sedmgt.pdf.

In the majority of contaminated sediments there are occurring very complex mixtures of organic pollutants, often also associated to heavy metals, which can frequently act as inhibitors of microbial activities and/or sources of highly toxic and bioaccumulable biotransformation byproducts (e.g., methylmercury generated from the microbial methylation of mercury in surface sediments) (Lloyd and Lovley, 2001; Apitz et al., 2004). Further, the metabolism of several halogenated pollutants and high-molecular-weight hydrocarbons results in the accumulation of a number of potentially toxic, recalcitrant and often more water soluble (and therefore mobile) intermediates (Bedard and Quensen, 1995; Adriaens and Vogel, 1995; Haggblom et al., 2003). The uncertainties here are: a) how and how much does the coexistence of similar or different pollutants affect the biological fate of each single pollutant in terms of biotransformation rate and extent, breakdown mechanism and accumulation of transformation intermediates; and b) how and by how much do daughter products resulting from pollutant biotransformation contribute to the final sediment toxicity. There is a need to look not just at the disappearance of priority pollutants but also at the net changes in toxic effects in sediments before and after biological treatment. In addition, a very few studies have been aimed at understanding the interactions among microorganisms directly and indirectly involved in pollutant biodegradation in real sediments (Abraham et al., 2002).

Finally, new mixtures of chemicals have entered recently the large market of personal care products. Pharmaceuticals, hormones and a number of new endocrine disruptors are detected at growing concentrations (from ng to mg/l) in several aquatic systems (Lee et al., 2003; Pedersen et al., 2005). The uncertainties here are on a) our ability to detect and characterize them and their biotransformation products in sediments in a reliable manner through the currently available analytical procedures and b) on their potential biodegradability and degradation pathways in aerobic and anaerobic sediments.

2.2. UNCERTAINTIES ON THE APPLICATION/EXPLOITING OF MICROBIAL CAPACITIES IN THE RESTORATION OF CONTAMINATED SEDIMENTS, IN PARTICULAR UNDER IN SITU CONDITIONS

The majority of information available on the biotransformation of pollutants in sediments and on the microorganisms potentially responsible for it has come from studies performed in laboratory-scale microcosms very often developed with sediments spiked with well defined target pollutants and suspended in synthetic culture media (Bedard and Quensen, 1995; Adriaens and Vogel, 1995; Haggblom et al., 2003). Thus, little is known about the biological fate of several priority pollutants in real sediments under actual site geochemical conditions. In addition, a very few studies have been aimed at a) investigating the occurrence and the main features in marine sediments of several biodegradation processes already elucidated in freshwater sediments, which markedly differ in many geochemical and biological properties from the marine ones (Haggblom et al., 2003; Apitz et al., 2004), b) developing strategies/approaches to stimulate pollutant biodegradation processes in sediments and c) understanding the interactions between microorganisms directly and indirectly involved in the pollutant biodegradation and the eukaryotic organisms, such as benthic organisms, co-occurring in real sediments.

In any case, laboratory-scale evidence does not prove that biodegradation will provide sufficient natural attenuation at the site (Apitz et al., 2004). Indeed, the coexistence of several different known and unknown pollutants along with the occurrence of lower and variable temperatures, (bio)turbation phenomena, etc, in the actual site sediment might markedly affect the occurrence and the route of biotransformation processes under *in situ* conditions (Bedard and Quensen, 1995; Adriaens and Vogel, 1995; Apitz et al., 2004). A lack of integrated metabolic knowledge within the *in situ* biogeochemical context might further contribute to making bioremediation processes unpredictable. Finally, tidal events, creeks, streams and the other features that move the sediments through transport may further adversely affect the onset and predictability of pollutant biodegradation *in situ*. Therefore, the main uncertainties are focused on a) how and to what extent the information currently available from laboratory experiments can be extrapolated and used for an effective prediction of microbial process potential and features *in situ*; b) how the spatial and temporal interactions between the large variety of native microorganisms with distinct metabolisms (reductive dechlorinators, methanogens, sulfate reducers, methanotrophic organisms) directly or indirectly involved in pollutant biodegradation can be managed and optimized in an *ex-situ* or an *in situ* treatment.

3. Needs in the area of biological treatment of contaminated sediments

Too much laboratory-scale research has been performed on spiked sediments suspended in artificial mineral media (Bedard and Quensen, 1995; Haggblom et al., 2003). We need studies performed on real contaminated sediments suspended in their own real water under laboratory conditions that closely mimic those occurring *in situ* or those under which the sediments are subjected to *ex-situ* treatment (Apitz et al., 2004). This might allow us to collect information of some relevance for predicting, when combined to lines of biogeochemical evidences (Apitz et al., 2004), the actual potential of biological processes in the final *in situ* restoration of contaminated sediments.

A lot of work needs to be done under these laboratory-scale conditions in order to collect reliable information on a) the rate, extent and mechanism of biodegradation of aged priority pollutants occurring in a representative number of both freshwater and marine sediments, and b) how these parameters might change by enhancing the bioavailability of pollutants through the increase (*via* spiking) of the concentration of these same pollutants in the same sediments or through the addition of specific nutrients, electron donors/acceptors, specialized microorganisms, etc. The microcosms thus developed need to be monitored through an integrated chemical, molecular and ecotoxicological analytical methodology, able to provide holistic information on a) the fate of the parent pollutants and of the metabolites generated from their biotransformation and the potential role of bioavailability on such parameters, b) the basic microbial processes (nitrate-, sulfate-, Fe(III) or Mn (IV)-consumption and volatile fatty acids, CH_4 or H_2 production) in progress in the sediment and the structure and key catabolic potential of indigenous microbial community and c) the toxicity of the sediment throughout the whole treatment.

These data, in turn, can be valuable in predicting: i) the potential fate of common mixtures of pollutants in different types of sediments, ii) the main background products that might be expected from their biodegradation and their impacts on the final sediment toxicity, iii) the nutrients or inocula useful for the process and iv) the possible dynamics through which the main members of the indigenous microbial community interact temporally and are potentially involved in the pollutant removal.

The last group of findings might be of great relevance in order to develop sediment-specific biostimulation or bioaugmentation strategies. However, because microbial metabolism in sediments is still poorly understood and only little information on key catabolic genes involved in such processes is available yet, for this aim there is a need to develop better, more robust monitoring tools, including molecular and other culture-independent approaches.

More knowledge on the interactions between indigenous microorganisms and benthic organisms might be also useful. The latter organisms can be responsible for an improved pollutant bioavailability, partial pollutant biodegradation and the establishment of geochemical conditions favorable for pollutant-degrading microorganisms. On the other hand, microorganisms might support benthic organisms by removing toxic pollutants, complementing their metabolism towards useful sediment substrates.

Laboratory scale investigations performed as described above can provide a body of reliable knowledge essential for a preliminary evaluation of the actual potential/relevance of an *in situ* natural recovery (NA/MNA) for a contaminated area. As stated above, such experimental evidence does not necessarily prove that biodegradation will provide sufficient natural decontamination of the site. However, it is a key line of evidence that, properly combined with other lines of microbial and biogeochemical evidence coming from the same site (Apitz et al., 2004), can permit the assessment of the role of biodegradation in the eventual natural recovery of the site.

Information obtained from microcosm studies performed with nutrients and inocula might be also useful in the preliminary design of a site-specific biostimulation/biaugmentation strategy. Here a lot of new knowledge is needed, in terms of commercially available nutrients, electron donors/acceptors, suitable inocula and the strategies/technologies through which to efficiently incorporate them into the contaminated sediment/water system.

The site conceptual modelling is also of great relevance for a reliable understanding/prediction of the susceptibility of a site to *in situ* natural recovery (Apitz et al., 2004). Here we need monitoring which not only provides qualitative descriptions of the *in situ* processes, but that also has the potential to supply parameter values for mathematical modelling of these processes. This is crucial in order to link conceptual understanding to performance models of biotreatment.

The hyporheic zone or interface between groundwater and surface water seems to play an important role in the natural attenuation of groundwater pollutants flowing from the sediment into surface water. This zone is a unique niche created by the sediment in which (bio)reductive processes can take place. For instance several chlorinated aliphatic hydrocarbons (PCE, TCE, etc) can be dehalogenated in this zone. Heavy metals such as Cd and Zn, can be precipitated as metal sulfides (formed by sulfate reducing bacteria, SRB). However some of these zones do have higher groundwater influx rates than others leading to shorter residence times. If the residence time is shorter than the time needed to perform the dehalogenation process, incompletely dehalogenated compounds are produced and enter the surface water. The need here is to develop site specific strategies, which might also consist of injecting redox manipulating compounds, to enhance the reactivity in the hyporheic zone.

Ex-situ treatment is successfully applied to bioremediation of PAH- and mineral oil-contaminated sediments under aerobic conditions (landfarming or bioreactors). The residual pollutant concentration represents a key limiting factor in this treatment and it is generally responsible for prolonged treatment times (Harmsen, 2004). Concentration limit values and standard guidelines for sediment reuse should be developed and applied at the international level.

Here more information on the possibility of detoxifying sediments through a biologically or chemically mediated pollutant binding to the sediment inorganic/organic components or combining biodegradation and catalyst-enhanced chemical degradation would contribute considerably to the development of improved or innovative highly effective *ex-situ* remediation strategies. Furthermore, to accomplish the same aim, it would be very useful to select biogenic, biodegradable and non-toxic pollutant "solubilizing" agents able to intensify in a totally biocompatible way the bioavailability and therefore the biodegradation of aged hydrophobic pollutants during sediment conventional *ex-situ* bioremediation (via bioreactor systems) or the pollutant mobilization during sediment washing operations (Abraham et al., 2002). This choice should be modulated by a prior assessment of the local microbial community, since, at least in some cases, addition of exogenous "solubilizing" or surfactant agents may interfere with the natural tendency of specific bacteria (e.g. *Mycobacteria*) to directly adhere on target pollutants (e.g. PAHs). The laboratory-scale microcosms approach described above should be the preferential strategy of choice for performing such investigations.

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