Interactions between metals, microbes and plants – Bioremediation of arsenic and lead contaminated soils

Riina Turpeinen

Department of Ecological and Environmental Sciences,
University of Helsinki

Academic dissertation in environmental ecology, to be presented, with the permission of the Faculty of Science of the University of Helsinki, for public criticism in Auditorium, Neopoli, Lahti,
on May 17th 2002, at 12 noon.

Lahti 2002

Supervised by

Prof. Max M. Häggblom
Rutgers, The State University of New Jersey
Department of Biochemistry and Microbiology
New Brunswick, NJ, USA

Prof. Timo Kairesalo
University of Helsinki
Department of Ecological and Environmental Sciences
Lahti, Finland

Dr. Mari Pantsar-Kallio
Environmental Specialist
UPM-Kymmene
Lahti, Finland

Reviewed by

Prof. Lee R. Krumholz
University of Oklahoma
Department of Botany and Microbiology
Norman, OK, USA

Dos. Kirsten Jørgensen
Finnish Environment Institute (SYKE)
Research Department
Helsinki, Finland

Opponent

Prof. Tamar Barkay
Rutgers, The State University of New Jersey
Department of Biochemistry and Microbiology
New Brunswick, NJ, USA

CONTENTS

PREFACE

The experimental part of this dissertation was carried out at the Department of Ecological and Environmental Sciences, University of Helsinki, Lahti, during the years 1998-2001. The work was financially supported by the University of Helsinki (project no. 2105018), the Maj and Tor Nessling foundation, Aboatox Oy (Turku, Finland), the Federation of Päijät-Häme, the City of Lahti, the Hollola commune, the Ministry of Education and the Academy of Finland (project no. 35522).

First, I want to thank my supervisor Professor Max Häggblom for his patient guidance and endless interest towards my work. I will always be thankful for the opportunity to visit Max´s lab in New Jersey. Max – you really made this thesis possible. I want to express my gratitude also to Professor Timo Kairesalo for inspiring discussions during these years and for support and help whenever needed. My sincere thanks as well to Dr. Mari Pantsar-Kallio who first awakened my interest in environmental chemistry and teached me the basics of scientific work. I will never forget "stimulating Sunny Beach Hotel".

I wish to thank all my co-workers. Especially, I thank M.Sc. Juha Lappalainen, M.Sc. Risto Juvonen (Aboatox Oy) and Dr. Marko Virta for introducing me to the world of biosensors; Dr. Loren Launen for taking care of me at Rutgers; Professor Lee Kerkhof for helping me with more or less imaginary problems with t-RFLPs; M.Sc. Anu El-Ghaoui, M.Sc. Anri Allonen (Lahti Research Laboratory) and Dr. Jari Tuominen, Head of the Lahti Research Laboratory, for sharing their knowledge of analytical chemistry with me and for help with metal analyses; Chief Engineer Antti Uusi-Rauva, Head of the Instrument Centre, and M.Sc. Tuomo Leppänen for help with radioactive work; laboratory worker Kimmo Järvinen for help with soil sampling and laboratory work; Professor Martin Romantschuk and Dr. Janne Salminen for critical reading of manuscripts; and Professor Jari Oksanen for helping me with the data-analyses.

I also want to express my sincere gratitude to Docent Kirsten Jørgensen and Professor Lee Krumholz for reviewing this thesis and giving constructive comments.

I am thankful for my close friends Hanna and Katja for friendship and care and for reminding me of life outside the laboratory. I wish to thank Hanna, Saku and Jussi for many crazy and refreshing "Jekku" happenings. To my Mom, Mummi and brothers Juho and Aapo I wish to express my most heartful gratitude for their support, love and encouragement. Finally, I thank Bama who brought joy into my life during the last nine years and meant everything to me.

ABSTRACT

Arsenic (As) and lead (Pb) are common inorganic toxicants at contaminated sites. Arsenic, like many other elements in soils, is subject to microbial transformations. For instance, reduction and methylation of arsenic by bacteria can form gaseous arsines leading to volatilization of arsenic or to mineralization of organic arsenic compounds to inorganic arsenic. These transformations influence arsenic cycling and accumulation in the soil. Accumulation of arsenic in soils is of great concern due to its toxicity and potential to contaminate groundwater. Also, because arsines are the most toxic forms of arsenic, their assessment in contaminated environment is important.

Assessment of mobility and bioavailability of lead in soils is also important as lead can pose significant groundwater or ecological risks in the environment. In lead contaminated soils, biota and vegetation influence the transformations of lead together with environmental parameters such as soil pH and organic matter content. In boreal forest soils, which are rich in humic material and have a podsolic stratification, lead contamination is mainly restricted to surface soil and is gradually passed down to the mineral horizon.

In order to increase the success of bioremediating contaminated soils, it is important to have a better understanding of how microbial populations and plants respond to elevated metal concentrations. In this study, the influence of microbes on the speciation of arsenic and production of arsines in contaminated soils was investigated under laboratory conditions. The volatilization process of arsenic was studied also in the field, in a landfill area where arsenic contaminated soils were temporarily stored. Because ionic species [in soils mainly as As(V)] may be mainly responsible for the biological effects of arsenic in soils, a previously developed luminescent As(III)-specific sensor bacterium was tested for its applicability for analysis of As(V) bioavailability in contaminated soil samples. Also, the effects of arsenic, chromium (Cr) and copper (Cu) contamination on the microbial community structure, potential microbial activity and arsenic-resistance were investigated in soils of long contaminated and abandoned wood impregnating plants. In addition, the effects of pine (*Pinus sylvestris*) on the solubility, mobility and bioavailability of lead in boreal forest soil were investigated in laboratory microcosms.

The results of this study revealed that microbes were able to carry out reactions resulting in changes in the speciation of arsenic in soil. The field results of the contaminated landfill area indicated that the concentrations of gaseous arsines may become high in the soil air, although the dominating species in soil was As(V). In addition, our experiments showed that leaching of arsenic to soil water may be largely driven by microbial activity. In contrast, pine seedlings were able to reduce the solubility, mobility and bioavailability of lead in contaminated boreal forest soil, indicating that pine has a major role in the immobilization of lead. Therefore, deep rooted plants, such as pine, can be used to reduce the risk of groundwater lead contamination through phytostabilization.

The phospholipid fatty acid (PLFA) and terminal restriction fragment (t-RFLP) analyses and microbial activity measurements indicated that microbes are able to adapt to arsenic, chromium and copper contamination and maintain the metabolic activity through changes in microbial community structure and selection for resistance. However, the bioavailability of arsenic was highly site-specific and for this reason similar inputs of arsenic had differing effects on soil microbial populations.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which in the text are referred to by their Roman numerals.

I Turpeinen, R., Pantsar-Kallio, M., Häggblom, M., Kairesalo, T. Influence of microbes on mobilization, toxicity and biomethylation of arsenic in soil. *The Science of the Total Environment* 236 (1999) 173-180.

II Turpeinen, R., Pantsar-Kallio, M., Kairesalo, T. Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils. *The Science of the Total Environment* 285 (2002) 133-145.

III Turpeinen, R., Virta, M., Häggblom, M.M. Analysis of arsenic bioavailability in contaminated soils. (submitted manuscript)

IV Turpeinen, R., Kairesalo, T., Häggblom, M.M. Microbial activity and community structure in arsenic, chromium and copper contaminated soils. (submitted manuscript)

V Turpeinen, R., Salminen, J., Kairesalo, T. Solubility, mobility and bioavailability of lead in contaminated boreal forest soil. *Environmental Science&Technology* 34 (2000) 5152-5156.

ABBREVIATIONS

As	arsenic, elemental
CA	sodium cacodylate
CCA	chromated copper arsenate
Cr	chromium, elemental
Cu	copper, elemental
CFU	colony forming units
DMA	dimethylarsine
DMAA	dimethyl arsinic acid
DOC	dissolved organic carbon
EC_{50} value	effective concentration giving 50 % inhibition of light output
FAME	fatty acid methyl ester
HG-AAS	hydride generation atomic absorption spectrometry
IC–ICP-MS	ion chromatography-inductively coupled plasma mass spectrometry
MMA	monomethylarsine
MMAA	monomethyl arsonic acid
Pb	lead, elemental
PLFA	phospholipid fatty acids
TMA	trimethylarsine
TMAO	trimethyl arsine oxide
t-RFLP	terminal restriction fragment length polymorphism

DEFINITION OF TERMS

The abbreviations As(III) and As(V) are used for arsenic in oxidation states +3 (arsenite) and +5 (arsenate).

The abbreviations for hydrated ionic forms are presented as charged species. For example, Pb^{2+} presents the hydrated form $[Pb(H_2O)_6]^{2+}$. As^{3+} presents the anions of arsenious acid and As^{5+} presents the anions of arsenic acid.

1. INTRODUCTION

1.1. Toxic metals in soil-microbe systems

1.1.1. Sources of metals in soils

Mineral rock weathering and anthropogenic sources provide two of the main types of metal inputs to soils. According to Ross (1994) the anthropogenic sources of metal contamination can be divided to five main groups: (1) metalliferous mining and smelting (arsenic, cadmium, lead and mercury); (2) industry (arsenic, cadmium, chromium, cobolt, copper, mercury, nickel, zinc); (3) atmospheric deposition (arsenic, cadmium, chromium, copper, lead, mercury, uranium); (4) agriculture (arsenic, cadmium, copper, lead, selenium, uranium, zinc); and (5) waste disposal (arsenic, cadmium, chromium, copper, lead, mercury, zinc). In Finland, most cases of soil metal contamination have been caused by waste treatment plants, sawmills, wood impregnation plants, shooting ranges, garages and scrap yards (Haavisto, 2002). In 2001, a total of 20 000 metal contaminated sites were identified (Haavisto, 2002). Because 38 % of these metal contaminated sites are located in groundwater areas or close to settled areas, metal contaminated soil sites are of great concern (Haavisto, 2002).

1.1.2. Toxic metals and toxicity mechanisms

Metals play an integral role in the life processes of microorganisms. Some metals, such as calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc, are essential, serve as micronutrients and are used for redox-processes; to stabilize molecules through electrostatic interactions; as components of various enzymes; and for regulation of osmotic pressure (Bruins et al., 2000). Many other metals have no biological role (e.g. silver, aluminium, cadmium, gold, lead and mercury), and are nonessential (Bruins et al., 2000) and potentially toxic to microorganisms. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions (Nies, 1999; Bruins et al., 2000). For example, Hg^{2+}, Cd^{2+} and Ag^{2+} tend to bind to SH groups, and thus inhibit the activity of sensitive enzymes (Nies, 1999). In addition, at high levels, both essential and nonessential metals can damage cell membranes; alter enzyme specificity; disrupt cellular functions; and damage the stucture of DNA (Bruins et al., 2000).

To have a physiological or toxic effect, most metal ions have to enter the microbial cell. Many divalent metal cations (e.g. Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) are structurally very similar. Also, the structure of oxyanions such as chromate resembles that of sulfate, and the same is true for arsenate and phosphate. Thus, to be able to differentiate between structurally very similar metal ions, the microbial uptake systems have to be tightly regulated. Usually, microorganisms have solved this problem by using two types of uptake systems for metal ions. One is fast, unspecific, and driven by the chemiosmotic gradient across the cytoplasmic membrane of bacteria (Nies, 1999). Since this mechanism is used by a variety of substrates, it is constitutively expressed (Nies, 1999). The second type of uptake system has a high substrate specificity, is slower, often uses ATP hydrolysis as the energy source and is only produced by the cell in times of need, starvation or a special metabolic situation (Nies and Silver, 1995).

Even though microorganisms have specific uptake systems, high concentrations of nonessential metals may be transported into the cell by a constitutively expressed unspecific system. This “open gate” is the one reason why metal ions are toxic to microorganisms (Nies, 1999). As a consequence, microorganisms have been forced to develop metal-ion homeostasis factors and metal-resistance determinants (Nies and Silver, 1995; Nies, 1999; Bruins et al., 2000). Because metal ions cannot be degraded or modified like toxic organic compounds, there are six possible mechanisms for a metal resistance system: exclusion by permeability barrier; intra- and extra-cellular sequestration; active efflux pumps; enzymatic

reduction; and reduction in the sensitivity of cellular targets to metal ions (Ji and Silver, 1995; Nies and Silver, 1995; Nies, 1999; Rensing et al., 1999; Bruins, 2000). One or more of these resistance mechanisms allows microorganisms to function in metal contaminated environments.

The oxyanions of arsenic enter bacterial cells via transporters for other compounds. In bacteria, As(V) is taken up by phosphate transport systems, such as the ATP-coupled Pst pump (Rensing, 1999). One route of entry for As(III) is via the GlpF polyol transporters (Rensing, 1999). The bacterial detoxification of arsenic is often based on inducible ion efflux systems that reduce the intracellular concentration of arsenic by active export (Ji and Silver, 1995; Nies and Silver, 1995; Rensing, 1999) (Fig. 1). Since anion export from bacterial cells is driven by the chemiosmotic gradient, simple As(III) efflux systems are composed of just one efflux protein (Ji and Silver, 1995; Nies and Silver, 1995; Rensing et al., 1999). As(V) cannot, however, be transported with this system. The solution to the problem of As(V) efflux is the enzyme arsenate reductase, which catalyzes the reduction of As(V) to As(III), the substrate of the efflux system (Ji and Silver, 1995; Nies and Silver, 1995; Rensing et al., 1999). Thus, this enzyme extends the spectrum of resistance to include both As(III) and As(V). The lead resistance may also be based on metal ion efflux. For example, zinc and cadmium specific pumps are able to export lead from bacterial cells (Nies, 1999; Rensing et al., 1999). In addition, lead resistance can be due to precipitation of lead phosphate within the cells of resistant bacteria (Nies, 1999).

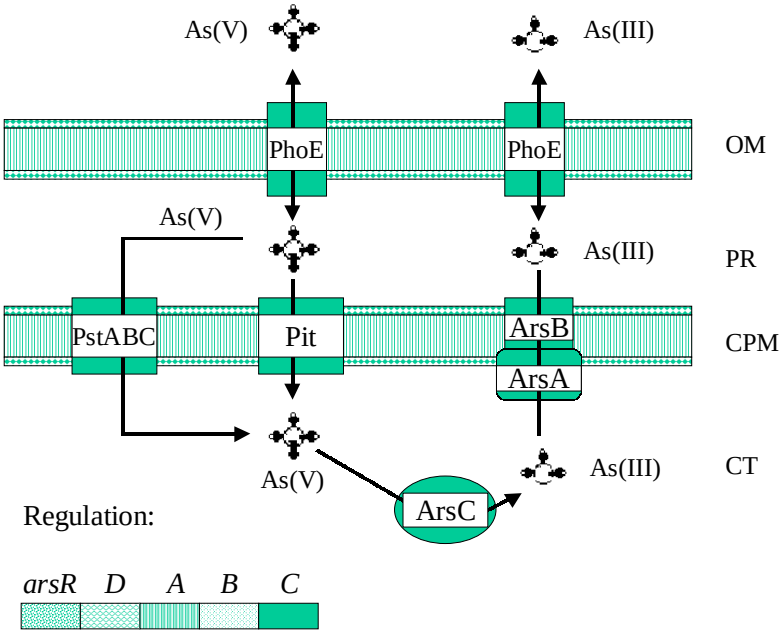

Fig. 1. Transport of and resistance to As(V) in *Escherichia coli*. As(V) enters the periplasmic space through the outer membrane porin, the PhoE protein, and is transported into the cytoplasm by the Pit protein or the Pst system. Within the cell, As(V) is reduced to As(III) by the ArsC protein and As(III) is pumped out of the cell by the ArsAB efflux ATPase. The arsRDABC operon is regulated by the ArsR repressor protein and ArsD co-regulator protein. OM=outer membrane, PR= periplasmic space; CPM= cytoplasmic membrane, CT cytoplasmic space. (Modified from Nies and Silver, 1995).

1.1.3. Biosensors

The genes responsible for microbial metal resistance mechanism are organized in operons and are usually found in plasmids carried by the resistant bacteria (Ramanathan et al., 1997; Bruins et al., 2000). The expression of the resistance genes is tightly regulated and induced

by the presence of specific metals in the cellular environment (Ramanathan et al., 1997). Because of the specificity of this regulation, the promoters and regulatory genes from these resistance operons can be used to construct metal-specific biosensors (promoter-reporter-gene fusions). By using metal specific bacterial sensors in addition to chemical analyses it is possible to distinguish the bioavailable metal concentration from the total metal concentration of the samples.

Recently, various metal-specific sensor strains have been developed and applied in many laboratories (Table 1). These sensor strains are all based on the same concept: a metal responsive regulation unit regulates the expression of a sensitive reporter gene. Reporter genes include those that code for bioluminescent proteins, such as bacterial luciferase (*luxAB*) and firefly luciferase (*lucFF*) or for β-galactosidase, which can be detected electrochemically or by using chemiluminescent substrates (Ramanathan et al., 1997; Cobisier et al., 1999; Bontidean et al, 2000). The light produced can be measured by a variety of instruments, including luminometers, photometers and liquid-scintillation counters.

Table 1. Recently developed bacterial sensors for metals.

METAL	REPORTER	HOST STRAIN	REFERENCE
Antimony (Sb^{3+})	*Luc*	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli*	Tauriainen et al., 1997
Arsenic (As^{3+})	*Luc*	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli*	Tauriainen et al., 1997, 1999
Arsenic (As^{3+})	*Lux*	*Escherichia coli*	Corbisier et al., 1993
Arsenic (As^{3+})	*Luc*	*Escherichia coli, Pseudomonas fluorescens*	Petänen et al., 2001
Arsenic (As^{5+})	*Lux*	*Escherichia coli*	Cai and DuBow, 1997
Arsenic (As^{5+})	*Luc*	*Escherichia coli*	This thesis
Cadmium (Cd^{2+})	*Luc*	*Staphylococcus aureus, Bacillus subtilis*	Tauriainen et al., 1998
Cobolt (Co^{2+})	*Lux*	*Ralstonia eutropha*	Tibazarwa et al., 2001
Copper (Cu^{2+})	*Lux*	*Pseudomonas fluorescens*	Tom-Petersen et al., 2001
Lead (Pb^{2+})	*Luc*	*Staphylococcus aureus, Bacillus subtilis*	Tauriainen et al., 1998
Mercury (Hg^{2+})	*Lux*	*Escherichia coli*	Selifonova et al., 1993
Mercury (Hg^{2+})	*Luc*	*Escherichia coli*	Virta et al., 1995
Mercury (Hg^{2+})	*Lux*	*Escherichia coli, Pseudomonas putida*	Hansen and Sørensen, 2000
Mercury (Hg^{2+})	*Lac*	*Escherichia coli*	Hansen and Sørensen, 2000
Mercury (Hg^{2+})	*Gfp*	*Escherichia coli*	Hansen and Sørensen, 2000
Mercury (Hg^{2+})	*Luc*	*Escherichia coli, Pseudomonas fluorescens*	Petänen et al., 2001
Nickel (Ni^{2+})	*Lux*	*Ralstonia eutropha*	Tibazarwa et al., 2001

In the construction of biosensors, it is important to know how metal-resistance mechanisms work and how the genes coding for them are regulated. For example, the resistance for mercury is encoded by genes of the *mer* operon (Ji and Silver, 1995; Osborn et al., 1997; Nies, 1999). This operon encodes proteins that are involved in the transport of mercury (Hg^{2+}) into the cell and for the transformation of Hg^{2+} to elemental mercury Hg^{0}, which is volatile and evaporates out of the cell (Nies, 1999). Selifonova et al. (1993) developed three separate biosensors containing different parts of the *mer* operon fused to the bacterial luciferase genes, using *E. coli* as a host strain. Two of the strains constructed did not contain the *merA* gene, which reduces Hg^{2+} to elemental mercury. Thus, these strains were not resistant to mercury and they could be used to detect only low concentrations of mercury. In contrast, the third strain contained the whole *mer* operon. This sensor was resistant to high concentrations of mercury but the response was not as sensitive as in the first two strains.

On the other hand, the *ars* operon of pR773, present in certain strains of *E. coli*, consists of five genes: *arsA, arsB, arsC, arsD* and *arsR*, which are responsible for arsenic resistance (Ji and Silver, 1995; Nies and Silver, 1995; Ramanthan et al., 1997). The *arsA* and *arsB* genes encode for the proteins that form the ion pump, which is capable of transporting As^{3+} out of the cell (Rensing et al., 1999). As^{5+} cannot be transported by this pump, but *arsC* encodes for an enzyme, arsenate reductase, that reduces As^{5+} to As^{3+}, which can then be removed by the pump (Ji and Silver, 1992). In addition, the *arsR* gene encodes for proteins that regulate the expression of the *ars* operon (Nies and Silver, 1995). In the arsenic biosensor developed by Tauriainen et al. (1997), the sensing of As^{3+} is based on controlling the expression of the firefly luciferase (*lucFF*) reporter gene by the regulatory unit of the *ars* operon of plasmid pR733 in recombinant plasmid pTOO31, with *E. coli* as a host strain. The regulatory unit of the *ars* operon consists of the *ars* promoter and the repressor protein, ArsR. In the absence of As^{3+}, the expression of *lucFF* is repressed, while in the presence of arsenic, transcription of the promoter is induced, and luciferase is produced at a level corresponding to the concentration of arsenic (Tauriainen et al., 1997).

In addition, the resistance mechanism for cadmium was utilized in the construction of a cadmium specific sensor bacterium (Tauriainen et al. 1998). The resistance for cadmium is encoded by the genes of the *cadA* operon, which consists of two genes: *cadA* and *cadC* (Yoon and Silver, 1991). The *cadC* gene encodes for the regulatory protein (Endo and Silver, 1995) and the *cadA* gene for an energy-dependent ion pump, which is responsible for efflux of cadmium from the cells (Tsai et al., 1992). In the sensor bacteria, plasmid pTOO24 carried the firefly luciferase reporter gene under the control of the *cad* promoter and the *cadC* gene of the *cadA* operon of *S. aureus* plasmid pI258 (Tauriainen et al., 1998). Because the sensor bacteria responded also to lead, it can be used to measure both bioavailable cadmium and lead.

1.1.4. Microbial transformations

Microbial transformations of metals serve various functions. Generally, microbial transformations of metals can be divided into two broad categories: redox conversions of inorganic forms; and conversions from inorganic to organic form and *vice versa*, typically methylation and demethylation. Through oxidation of iron, sulfur, manganese and arsenic, microbes can obtain energy (Tebo et al., 1997; Santini et al., 2000). On the other hand, reduction of metals can occur through dissimilatory reduction where microorganisms utilize metals as a terminal electron acceptor for anaerobic respiration. For example, oxyanions of arsenic (Stolz and Oremland, 1999; Niggemyer et a., 2001), chromium (QuiIntana et al., 2001), selenium (Stolz and Oremland, 1999) and uranium (Tebo and Obraztsova, 1998) can be used in microbial anaerobic respiration as terminal electron acceptors. In addition, microorganisms may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance. For example, aerobic and anaerobic reduction of Cr(VI) to Cr(III) (QuiIntana et al., 2001; Nkhalambayausi-Chirwa and Wang,

2001); reduction of Se(VI) to elemental selenium (Lloyd et al., 2001); reduction of U(VI) to U(IV) (Francis, 1998; Chang et al., 2001); and reduction of Hg(II) to Hg(0) (Brim et al., 1999; Wagner-Döbler et al., 2000) are widespread detoxification mechanisms among microorganisms.

Microbial methylation plays an important role in the biogeochemical cycle of metals, because methylated compounds are often volatile. For example, mercury [Hg(II)] can be biomethylated by a number of different bacterial species (e.g. *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp. and *Clostridium* sp.) to gaseous methylmercury (Pan-Hou and Imura, 1982; Compeau and Bartha, 1985; Pongratz and Heumann, 1999), which is the most toxic and most readily accumulated form of mercury (Nikunen et al., 1990). Also, biomethylation of arsenic to gaseous arsines (Gao and Burau, 1997); selenium to volatile dimethyl selenide (Flury, 1997; Guo et al., 1999; Martens and Suarez, 1999; Zhang and Frankenberger, 1999; Dungan and Frankenberger, 2000); and lead to dimethyl lead (Pongratz and Heumann, 1999) has been observed in various soil environments.

In addition to redox-conversions and methylation reactions, acidophilic iron- and sulfur-oxidizing bacteria are able to leach high concentrations of arsenic, cadmium, copper, cobolt, nickel and zinc from contaminated soils (White et al., 1997; Seidel et al., 2000; Groudev et al., 2001; Löser et al., 2001). On the other hand, metals can be precipitated as insoluble sulfides indirectly by the metabolic activity of sulfate-reducing bacteria (White et al., 1997; Lloyd et al., 2001). Sulfate-reducing bacteria are anaerobic heterotrophs utilizing a range of organic substrates with SO_4^{2-} as the terminal electron acceptor.

In summary, microbiological processes can either solubilize metals, thereby increasing their bioavailability and potential toxicity, or immobilize them, and thereby reduce the bioavailability of metals. These biotransformations are important components of biogeochemical cycles of metals and may also be exploited in bioremediation of metal contaminated soils (Lovley and Coates, 1997; Gadd, 2000; Barkay and Schaefer, 2001; Lloyd and Lovley, 2001).

1.1.5. Microbial activity and community structure in metal contaminated soils

Even though many soil microbes are able to carry out various transformations of metals, high concentrations of metals can harmfully effect the soil microbial activity and functioning. Recently, numerous laboratory and field studies have demonstrated the adverse effect of metals on the soil ecosystem. Significant reductions in microbial biomass (Frostegård et al., 1993; Fliessbach et al., 1994; Roane and Kellogg, 1996; Konopka et al., 1999) and soil respiration (Doelman and Haanstra, 1984; Bååth et al., 1991; Hattori, 1992) have been found in metal contaminated soils compared to uncontaminated soils. Also, many studies have shown that metal contamination causes a shift within the soil microbial community from sensitive to less sensitive microbes (Malizewska et al., 1985, Capone et al., 1983; Bååth et al, 1989; Said and Lewis, 1991; Roane and Kellogg, 1996; Dahlin et al., 1997; Bååth et al., 1998a, 1998b; Khan and Scullion, 2000).

On the other hand, to resist the toxic effects of metals, microorganisms have evolved different resistance mechanisms to avoid metal toxicity, as discussed earlier (Ji and Silver, 1995; Nies and Silver, 1995; Nies, 1999; Bruins et al., 2000). As a consequence, the replacement of sensitive species by resistant species may not result in any net effect on general microbial sum parameters such as soil respiration or total biomass (Bååth, 1989). For example, Bardgett and Saggar (1994) and Fliessbach et al. (1994) reported increased CO_2 evolution in metal-polluted soils. Adaptation is thus an important mechanism behind the responses of microbes to the presence of soil contaminants (Doelman, 1986; Chew et al., 2001; Muller et al., 2001) and may result in the compensation of an adverse effect by the increased activity of the remaining microbiota (Duxbury and Bicknell, 1983).

The dissimilar findings of microbial responses to soil metal contamination may also have resulted from variations in the levels of metal contamination; in the source of the contamination; in the period of time over which the responses were monitored; in characteristics of the recipient soil (Khan and Scullion, 2000); and in variations of metal bioavailability (Roane and Kellogg, 1996). In addition, the discrepancy in microbial community studies may be attributed to the methods used earlier, which provide data on community processes or culturable bacterial numbers and may not be relevant for a more detailed analysis of microbial community structure or diversity. Because most of these methods require the use of media or substrates which are selective for culturable organisms, the majority of soil microorganisms are usually not detected (Faegri et al., 1977; Smit et al., 1997; Sandaa et al., 2001).

Recent advances in molecular fingerprinting methods using signature biomarkers, such as lipids and nucleic acids, provide a qualitative and quantitative measurement of microbial diversity and community composition in undisturbed and polluted soils (White et al., 1998; Torsvik et al., 1998; Kozdroj and van Elsas, 2001). For example, phospholipid fatty acid analysis (PLFA) can be used to quantify microbial community structure and biomass without relying upon the cultivaltion of microorganisms (Kozdroj and van Elsas, 2001). Unfortunately, this approach does not have the capability of identifying microorganisms at the species or strain level, but rather produces descriptions of microbial communities based on functional groupings of fatty acid profiles. However, the identification of particular species, contributing to the bacterial community, by cell fatty acid methyl ester profiles (FAME) can be determined from cultured isolates (Zelles, 1997).

Changes in the microbial community structure in response to soil metal contamination has been monitored by PLFA analysis in various studies. For example, accumulation of copper in soil as a concequence of fungicide application resulted in the development of a microbial community with markedly different PLFA patterns when compared to uncontaminated soil (Zelles et al., 1994). In metal contaminated soils, the increase of monounsaturated fatty acids, lower concentrations of branched-chain and methyl-branched fatty acids indicated an increase in numbers of gram-negative bacteria and a decrease of the proportion of gram-positive bacteria and actinomycetes, respectively. Also, in studies of Hiroki (1992) and Frostegård et al. (1993) a decrease in several iso- and anteiso-branched PLFAs and an increase in cy17:0, which is considered to be typical for gram-negative bacteria (Lechevalier, 1977), indicated a dominance of gram-negative over gram-positive bacteria in metal contaminated soils. However, Pennanen et al. (1996) found that many branched PLFAs, like br17:0 and br18:0, or iso- and anteiso-branched PLFAs, commonly found in gram-positive bacteria, increased in the metal contaminated soil. Similar results were found also in coniferous forest humus due to Ni-Cu pollution and acidification (Pennanen et al., 1998). In coniferous forest soil, also fungal markers (18:2ω6,9 and 20:4) decreased in response to long-term heavy metal deposition (Pennanen et al., 1996), even though various studies have found that fungi are more resistant to metals than bacteria (Frostegård et al., 1993; Lechevalier, 1975; Hiroki, 1992; Khan and Scullion, 2000). According to Pennanen et al. (1996, 1998), these contradictory findings could be due to a reduction in the forest floor vegetation and rhizosphere habitats in metal contaminated soils, which are typically dominated by gram-negative bacteria. On the other hand, the methyl-branched PLFAs 10Me16:0, 10Me17:0 and 10Me18:0 increased in metal-polluted forest soil, indicating an increase in the proportion of actinomycetes (Frostegård et al., 1993). However, in arable soils, a decrease was observed for 10Me16:0 and 10Me18:0 in response to most metals (Frostegård et al., 1993). Also, Kelly et al. (1999) reported of a relative decrease in 10Me16:0 in zinc contaminated soil when compared to undisturbed soil, suggesting that different members of the actinomycete population responded differently to the elevated metal cocentrations.

A molecular approach based on 16S rDNA is useful in detecting bacterial community structure changes, because these genes are conserved and present in all bacteria (Moyer et al., 1994). Microbial community analyses using nucleic acids, such as 16S rDNA, can detect and identify community members with high specificity to the species and strain level, and can also detect and suggest phylogenetic relationships of uncultured organisms (Sakano and Kerkhof, 1998; Phelps et al., 1998; Marsh, 1999; Häggblom et al., 2000; Kerkhof et al., 2000). For example, Smit et al. (1997) and Torsvik et al. (1998) using amplified ribosomal DNA restriction analysis (ARDRA) found distinct differences in microbial community structure in soil contaminated with heavy metals compared to uncontaminated soil. An alternative 16S rDNA-based method is terminal restriction fragment analysis (t-RFLP), which measures only the terminal restriction fragment of each 16S rRNA gene (Marsh, 1999). Thereby, the complexity of the RFLP pattern is reduced (compared to that of ARDRA) and every visible band (fragment) is representative of a single ribotype or operational taxonomic unit (Liu et al., 1997; Marsh, 1999). For example, Widmer et al. (2001) compared microbiological characteristics of three soils contaminated with pesticides and Clement et al. (1998) were able to distinguish the microbial community in hydrocarbon contaminated soil from control soil by t-RFLP.

1.2. Arsenic in the environment

Although arsenic has almost exclusively been assosiated with criminal poisoning for many centuries, the matter of concern today is its contribution to environmental pollution through man´s use of arsenic containing insecticides, herbicides, fungicides, pesticides and wood preservatives and through mining and burning of coal (Leonard, 1991). Thus, anthropogenic use makes arsenic a common inorganic toxicant found at contaminated sites nationwide (Davis, 2001). Consequences of long-term exposure to inorganic forms of arsenic are serious because these compounds have been recognized as skin and lung carcinogens in humans. Because the toxic effects of arsenic are related to its oxidation state (Pongratz, 1998), determination of individual species of arsenic and the examination of factors affecting the speciation of arsenic have become an important issue during the past decade.

1.2.1. Arsenic species in soils

Arsenic (As) occurs naturally in a wide range of minerals. Also, mining activity and widespread use of arsenic in the wood preserving industry and in agriculture as a pesticide and herbicide represent a major source of arsenic in the environment. The common valence states of arsenic in nature include –3, 0, +3 and +5 (Leonard, 1991; Jain and Ali, 2000; Oremland et al., 2000). In soils, the most often encountered arsenic forms are inorganic As(III) (arsenite) and As(V) (arsenate) (Cullen and Reimer, 1989; Masscheleyn et al., 1991; Pantsar-Kallio and Korpela, 2000; Balasoiu et al., 2001). Methylated species, monomethyl arsonic acid (MMAA), dimethyl arsinic acid (DMAA) and trimethyl arsine oxide (TMAO), dominate in biomass, but have also been detected in soils (Buchet and Lauwerys, 1981; Leonard, 1991). In addition, As(V) and As(III) can be volatilized to arsine (AsH_3); MMAA to monomethylarsine (CH_3AsH_2; MMA); DMAA to dimethylarsine [$(CH_3)_2AsH$; DMA]; and TMAO to trimethylarsine [$(CH_3)_3As$), TMA] (Cullen and Reimer, 1989).

1.2.2. Biological properties of arsenic and its compounds

The impacts of arsenic on biological systems are concentration dependent and vary from organism to organism. In general, the toxicity of arsenic is dependent on its oxidation state: trivalent arsenic forms are approximately 100 times more toxic than the pentavalent derivatives (Cervantes, 1994). Toxicity of As(III) is due to its binding to protein sulfhydryl groups (Gebel, 2000). Thus, As(III) inhibits enzyme reactions requiring free sulfhydryl groups, leading to membrane degradation and cell death. As(V), on the other hand, is a toxic analog for inorganic phospate in phosphorylating metabolism (Cervantes, 1994).

As(V) competes with phosphate and therefore acts as an uncoupler of oxidative phosphorylation, resulting in inadequate supply of energy. In general, methylated species, MMAA and DMAA, are less toxic forms than inorganic As(V) and As(III) because of their low solubility and reduced affinity to tissues (Leonard, 1991). For plants, however, organic arsenic compounds are highly toxic when applied foliarly (Marin et al., 1992, 1993). For more developed organisms, gaseous arsines are the most toxic forms of arsenic (Buchet and Lauwerys, 1981; Leonard, 1991) due to their ability to combine with hemoglobin within the red blood cells, causing destruction or severe swelling of the cells and rendering them nonfunctional (Blair et al., 1990).

1.2.3. Influence of microbes on the speciation and mobility of arsenic

Microorganisms play an important role in the environmental fate of arsenic with a multiplicity of mechanisms affecting transformations between soluble and insoluble arsenic forms and toxic and nontoxic arsenic forms. Inorganic arsenic forms, As(V) and As(III), are subjected to microbiologically mediated oxidation-reduction reactions. For example, a *Pseudomonas* strain (with the proposed name "*P. arsenitoxidans*") can derive metabolic energy from the oxidation of As(III) (Ilyaletdinov and Abdrashitova, 1981). *Thermus aquaticus* and *Thermus thermophilus* were also found to rapidly oxidize As(III) to As(V), but they were not able to grow with As(III) as the sole energy source, thus suggesting that the ecological role of As(III) oxidation was detoxification of arsenic (Gihring et al., 2001).

On the other hand, As(V) can be reduced by dissimilatory reduction, where microorganisms utilize As(V) as a terminal electron acceptor for anaerobic respiration. To date, dissimilatory reduction has been observed in several bacteria, such as *Sulfurospirillum barnesii, S. arsenophilum, Desulfotomaculum auripigmentum, Bacillus arsenicoselenatis, B. selenitireducens, Crysiogenes arsenatis, Sphingomonas* spp., *Pseudomonas* spp. and *Wolinella* spp. (Ahmann et al., 1994; Lovley and Coates, 1997; Newman et al., 1997; Newman et al., 1998; Stolz and Oremland, 1999; Oremland et al., 2000; Macur et al., 2001). In addition, microorganisms may possess As(V) reduction mechanisms that are not coupled to respiration but instead are thought to impart arsenic resistance. For example, a plasmid-encoded, detoxifying reductase (*arsC* enzyme) present in the cytoplasm of certain bacteria (e.g. *Escherichia coli* and *Staphylococcus aureus*) reduces As(V) to As(III) for its rapid extrusion from the cell (Ji et al., 1994; Diorio et al., 1995). In addition, Gihring and Banfield (2001) isolated a new *Thermus* strain from an arsenic-rich terrestrial geothermal environment, which was capable of both As(III) oxidation and As(V) dissimilatory reduction.

Microbes are also able to biomethylate inorganic arsenic species to MMAA and DMAA (Ridley et al., 1977; Woolson, 1977a; Cullen and Reimer, 1989; Gadd, 1993). In contrast, demethylating microorganisms can carry out conversion of methylated species back to inorganic forms (Sohrin et al., 1997). In addition, microbial activity can result in volatilization of arsenic to gaseous arsines (Bachofen et al., 1995; Gao and Burau, 1997). Arsines may travel in air or they are oxidized rapidly depending on environmental conditions (Pongratz, 1998). Oxidation returns arsenic back to inorganic species, As(V) or As(III), or to organic forms, MMAA, DMAA or TMAO, and the cycle of arsenic is completed because atmospheric arsenic is deposited back to soil by rain or by dry deposition (Pongratz, 1998) as illustrated in Fig. 2. In summary, the basis for arsenic transformations in soils lies primarily in the microbiological methylation and oxidation-reduction processes.

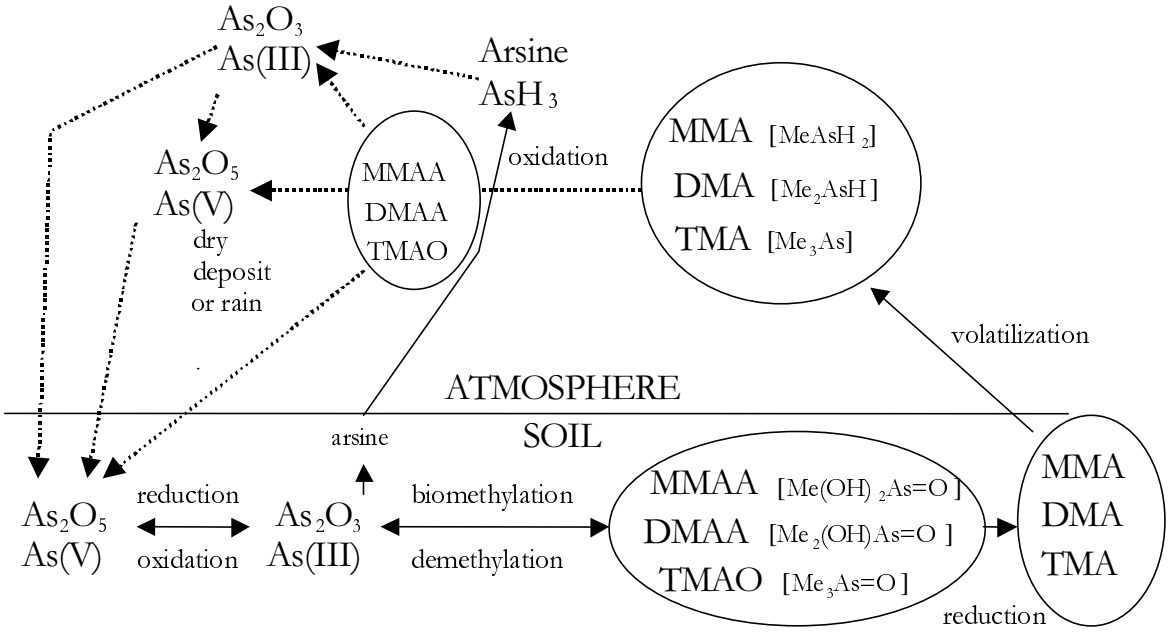

Fig. 2. Microbial (⟶) and abiotic (⇢) transformations of arsenic in soils. Particulate species are not presented in the figure. [MMAA=monomethyl arsonic acid; DMAA=dimethyl arsinic acid; TMAO=trimethyl arsine oxide; MMA=methylarsine; DMA=dimethylarsine; TMA=trimethylarsine.]

One example of microbial transformations of arsenic relates to the fate of the French emperor Napoleon Bonaparte. For a long time it was thought that Napoleon had died because of stomach cancer. However, recent scientific analyses of Napoleon´s hair have revealed arsenic levels from 35 up to 640 times (10-250 mg kg^{-1}) found in the hair of a healthy human suggesting that he had been poisoned with arsenic (Lewin et al., 1982). After this discovery, David Jones started to clarify the links between Napoleon´s death and arsenic and a few years later he reported that it seems quite obvious that arsine poisoning caused Napoleon´s death (Jones, 1982). Jones was fortunate enough to be able to obtain a piece of the original wallpaper from Longwood House, St. Helena, where Napoleon had been imprisoned. The wallpaper, infact, contained large amounts of arsenic due to use of a green pigment containing copper and arsenic. Furthermore, the house itself was very damp and it was necessary to change the wallpaper at frequent intervals due to the development of moulds. Thus, when the wallpaper became damp, moulds, such as *Penicillium previcaulis*, likely grew on the wallpaper using the cellulose or glue for growth. The moulds may have converted arsenic to the volatile and highly poisonous trimethylarsine that volatilized into the atmosphere of the room. Thus, the villain of the story may not have been only the British authorities, but rather the wicked wallpaper mould *Penicillium previcaulis*. Even though it is difficult to know the truth about Napoleon´s death afterwards, this story is a good example of unexpected problems caused by toxic metals and their species conversion.

Studies on arsenic transformations in soils have shown that the process is affected not only by the microorganisms involved but also by the soil evironment and by arsenic characteristics (Gadd, 1993). The presence of certain elements and ions, such as phosphate (Huysmans and Frankenberger, 1991 – inhibition), antimony (Andrewes et al., 2000 – inhibition) and molybdate (Oremland et al., 2000 – enhancement), may inhibit or enhance microbiological transformations of arsenic in soil systems. According to Gao and Burau (1997) soil properties such as pH, texture, temperature and organic matter content all affect the arsine evolution rate. In addition, a study of the evolution of gaseous arsenic forms in soils showed, that the production of arsines was enhanced under reduced, wet soil

conditions (Atkins and Lewis, 1976). Arsine evolution from arsenic-amended soil was dependent also on the speciation of arsenic. The cumulative arsine production followed the order CA > MMAA > As(III) > As(V), indicating that evolution of arsines is much higher from organic arsenic compounds than from inorganic arsenic compounds (Gao and Burau, 1997).

1.2.4. Bioavailability of arsenic in contaminated soils

The toxicological effects of arsenic depend on its chemical form and bioavailability (La Force et al., 2000). Therefore, hydrated ions are considered to be the most toxic forms of arsenic, while strong complexes and species associated with colloidal particles are usually assumed to be less toxic (Russeva, 1995).

In soils, the bioavailability and thus toxicity of arsenic is dependent on various soil parameters. For example, waterlogging, pH, redox-conditions, other elements, soil and site hydrology and plant and microbial components influence the adsorption capacity and behaviour of soil colloids (clay, oxides or hydroxides, surfaces of aluminium, iron and manganese, calcium carbonates and/or organic matter), and thus alter the solubility and bioavailability of arsenic (Woolson, 1977b; Sadiq, 1997). In general, iron oxides/hydroxides are most commonly involved in the adsorption of arsenic in both acidic and alkaline soils (Polemio et al., 1982). In the acidic oxic and suboxic soils, Fe-arsenate [$Fe_3(AsO_4)_2$] may control arsenic solubility, whereas in anoxic soils, sulfides of As(III) may control the concentrations of the dissolved arsenic in soil solutions (Sadiq, 1997). According to Hongshao and Stanforth (2001), also direct precipitation as discrete arsenic solid phases [i.e. as reduced phases such as arsenopyrite (FeAsS) or as oxidized phases such as hematite (Fe_2O_3) associated with arsenic] may occur in contaminated soils. This indicates that as arsenic persists, or ages, it becomes progressively less soluble and bioavailable with time (Alexander, 2000).

Generally, due to sorption reactions and solid phase formation of arsenic in contaminated soils, the soil particulate phases have high concentrations of arsenic relative to the concentrations of arsenic in dissolved phase or in terrestrial organisms (Peijnenburg et al., 1997; Alexander, 2000). Traditional methods used to evaluate the risk of contaminants in an ecosystem include the analyses of soil total metal concentrations (Alexander, 2000; Murray et al., 2000). The relationship between the total metal content in the soil and its effect on biota is not, however, straightforward (Plette et al., 1999). As a consequence, current approaches for ranking sites for cleanup may lead to choosing less acute sites for remediation resulting in delaying cleanup of such polluted areas where the risk is greater (Alexander, 2000). Also, even though microbial activity measurements have proven to be good indicators of the degree of pollution of metal contaminated soils (Tabatabai, 1977; Bååth et al., 1992; Insam et al., 1996), possible adaptation of the microbial communities in contaminated soils may induce a significant bias in this evaluation (Giller et al., 1998). Therefore, the ability to distinguish bioavailable arsenic from the total metal content is important.

The bacterial-based biosensors can be used to assess the bioavailability of arsenic. For example, Tauriainen et al. (2000) used biosensors to detect As(III) in natural water samples. In addition, Cai and DuBow (1997) applied a bacterial sensor to estimate the toxicity of arsenic-containing wood preservative, chromated copper arsenate. The use of biosensors in assessing the bioavailability of arsenic in soil samples is, however, complicated. The response of the biosensor may be biased by the presence of the soil particles. On the other hand, filtration to remove soil particles may result in an underestimation of arsenic bioavailability, because some of the arsenic fractions, for example arsenic complexes with

colloidal and fine particles, are eliminated in filtration. These fractions can, however, be important factors when considering the toxicity of arsenic in soils.

1.3. Lead in the environment

1.3.1. Properties of lead

In nature, lead (Pb) is a ubiquitous but biologically non-essential element (Nriagu, 1978; Ewers and Schlipköter, 1991). However, during the last fifty years the use of lead in batteries, bearing metals, cable covering, gasoline additives, explosives and ammunition as well as in manufacture of pesticides, antifouling paints and analytical reagents has caused widespread environmental contamination (Ewers and Schlipköter, 1991; Watanabe, 1997; Johnson, 1998). The toxicity of lead is a consequence of the ability of Pb^{2+} to interfere with several enzymes (Ewers and Schlipköter, 1991). Because lead causes a large variety of toxic effects, including gastrointestinal, muscular, reproductive, neurological and behavioral and genetic malfunctions (Johnson, 1998), the fate of lead in the environment is of great concern.

In lead-contaminated soils, biota and vegetation influence the transformations of lead together with environmental characteristics such as soil pH, organic matter content, texture, redox-potential and presence of other elements (van der Sloot et al., 1996). According to Bindler et al. (1999) lead contamination is mainly restricted to surface soil in boreal forests, which are rich in humic material and have a podsolic stratification. Also, Heinrichs and Mayer (1980) considered lead as one of the least soluble metals with a very long retention time in the forest floor. However, Friedland et al. (1992) found that lead complexed with dissolved organic matter may migrate from the surface soil layer to mineral soil, thus raising the concern of lead contaminating the groundwater. Also, Johnson et al. (1995) reported that concentrations of lead in humic surface soils have declined between 1977 and 1987 by 29 %, thus indicating that lead had passed down to the mineral soil. In addition, Ulkomaanaho et al. (2001) found that the retention of lead by the humus layer of boreal forest soil was only 26-54 % of the total input of lead to the forest floor. Therefore, cleaning up lead-contaminated soils or detoxifying lead with the fewest environmental side effects is of great interest and practical methods are needed.

1.3.2. *Phytoremediation of lead*

Traditional methods of remediating lead contaminated sites include a variety of physical, thermal and chemical treatments (Berti and Cunningham et a., 1997; Chen et al., 2000; Mulligan et al., 2001). Recently, also phytoremediation technologies have been recognized as quite efficient and cost-effective method for remediating sites contaminated with toxic metals (Salt et al., 1995; Shann, 1995; Chaney et al., 1997; Raskin et al., 1997; Meagher, 2000; Garbisu and Alkorta, 2001; van der Lelie, 2001). Categories of phytoremediation include:

1. phytoextraction - the use of plants to remove contaminants from soil
2. phytovolatilization - the use of plants to make volatile chemical species of soil elements
3. phytostabilization - the use of plants to transform soil metals to less toxic forms, or to reduce mobility, but not remove the metal from soil.

For phytoextraction to be a feasible remediation tool, plants that are used have to be able to take up large concentrations of heavy metals into the roots; to translocate these metals to the shoots; and to grow rapidly and reach a high biomass, which can be harvested (Kumar et al., 1995; Mejare and Bulow, 2001). For lead, there are a few plants, such as *Brassica juncea* (Watanabe, 1997), *Vetiveria zizanioides* (Chen et al., 2000), *Cardaminopsis halleri*

(Dahmani-Muller et al., 2000), *Spartina alterniflora* (Windham et al., 2001), *Cynodon dactylon* and *Sorghum halepense* (Madejon et al., 2001), that possess these qualities. However, according to Reimann et al. (2001) none of the tested plants growing in Northern Europe (blueberry, *Vaccinium myrtillus*; cowberry, *Vaccinium vitis-idaea*; crowberry, *Empetrum nigrum*; birch, *Betula pubescens*; willow, *Salix* spp.; pine, *Pinus sylvestris*; and spruce, *Picea abies*) accumulated lead effectively into their shoots, thus not enabling efficient phytoextraction of lead. To overcome these shortfalls, the use of metal chelators such as EDTA have been applied to soils to make lead more bioavailable for plant uptake. In several experiments, the use of these chelators has dramatically increased the amount of lead that high biomass plants take into their roots and translocate to their shoots (Huang and Cunningham, 1996; Huang et al., 1997; Blaylock et al., 1997; Cooper et al., 1999). The use of the chelators in the phytoextraction process, however, creates problems: remediation costs are increased and metals that are mobilized by the chelators can migrate offsite and contaminate e.g. underlying groundwater (Brennan and Shelley, 1999; Römkens et al., 2002).

Even though no real lead accumulator plant growing in Northern Europe has been identified, high concentrations of lead have been found in the roots of pine (Hartley et al., 1999), one of the dominant tree species in boreal forests. In addition, the concentrations of lead were much higher in the roots of *Agrostis tenuis* (Dahmani-Muller et al., 2000) *Arabidopsis halleri* (Dahmani-Muller et al., 2001), *Typha latifolia* and *Scirpus sylvaticus* (Hozhina et al., 2001) than in the shoots suggesting that lead was immobilized by roots of various plants. Also, Klassen et al. (2000) have found that *Betula occidentalis* promoted soil lead stabilization by sequestering mobile lead fractions in its rhizosphere.

The inherent hazard posed by lead contained in a soil matrix is a function of its relative mobility and bioavailability, which are dependent on soil characteristics such as pH, mineralogy, texture, organic matter content as well as on the source and quantity of lead in the soil (Berti and Cunningham, 1997). Therefore, phytostabilization, i.e. the use of metal-tolerant plant species to immobilize contaminants in the soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the rhizosphere, appears to be a potential remediation technique for lead contaminated soils (Huang and Cunningham, 1996; Berti and Cunningham, 1997; Chaney et al., 1997). This process reduces the mobility of the lead and prevents migration to the groundwater. Through phytostabilization it is also possible to reduce the bioavailability of lead and thus its entry into the terrestrial food webs.

2. AIMS OF THE RESEARCH

Microbes play a key role in controlling the speciation and cycling of metals in soil. Because bioavailability, toxicity and reactivity of metals is greatly influenced by chemical speciation, it is important to have a better understanding of the major factors that link microbial activity to the biogeochemistry of metals. In addition, plants can considerably alter the mobility and bioavailability of metals in soil. Therefore, understanding the roles of microorganisms and plants in cycling of metals may lead to improved processes for bioremediation of contaminated sites.

Arsenic and lead are toxic metals found at several contaminated sites. Thus, there is a high level of interest in developing methods aimed at cleaning up or detoxifying arsenic and lead contaminated soils at minimal costs with the fewest environmental side effects. To get some insight on how microbes and plants could be exploited in developing bioremediation methods for arsenic and lead contaminated soils, the specific aims of the research in this thesis were:

1. To determine the effects of microbial activity on the speciation and mobility of arsenic in soil. (Papers I and II)

2. To determine the influence of microbes on the production of arsines in arsenic-contaminated soils. (Paper II)

3. To measure the amount of bioavailable As(V), the dominant arsenic species in soils, using a luminescent arsenic-specific bacterial sensor. (Papers III and IV)

4. To characterize and compare the structure and function of microbial populations in arsenic-, chromium- and copper-contaminated soils. (Paper IV)

5. To examine the effects of pine and liming on the mobility and bioavailability of lead in boreal forest soil. (Paper V)

6. To evaluate the use of bioremediation in cleaning up or detoxifying arsenic- and lead-contaminated soils. (Papers III and V)

3. MATERIALS AND METHODS

This chapter provides a brief outline of the materials and methods used. More detailed descriptions are given in each original paper.

3.1. Sampling sites

Arsenic-contaminated soils were collected from seven different wood impregnating plants (Sites 1, 3-8) and from one landfill of contaminated soils (Site 2) (Fig. 3; Table 2). Lead-contaminated soil was collected from a shooting range (Site 9).

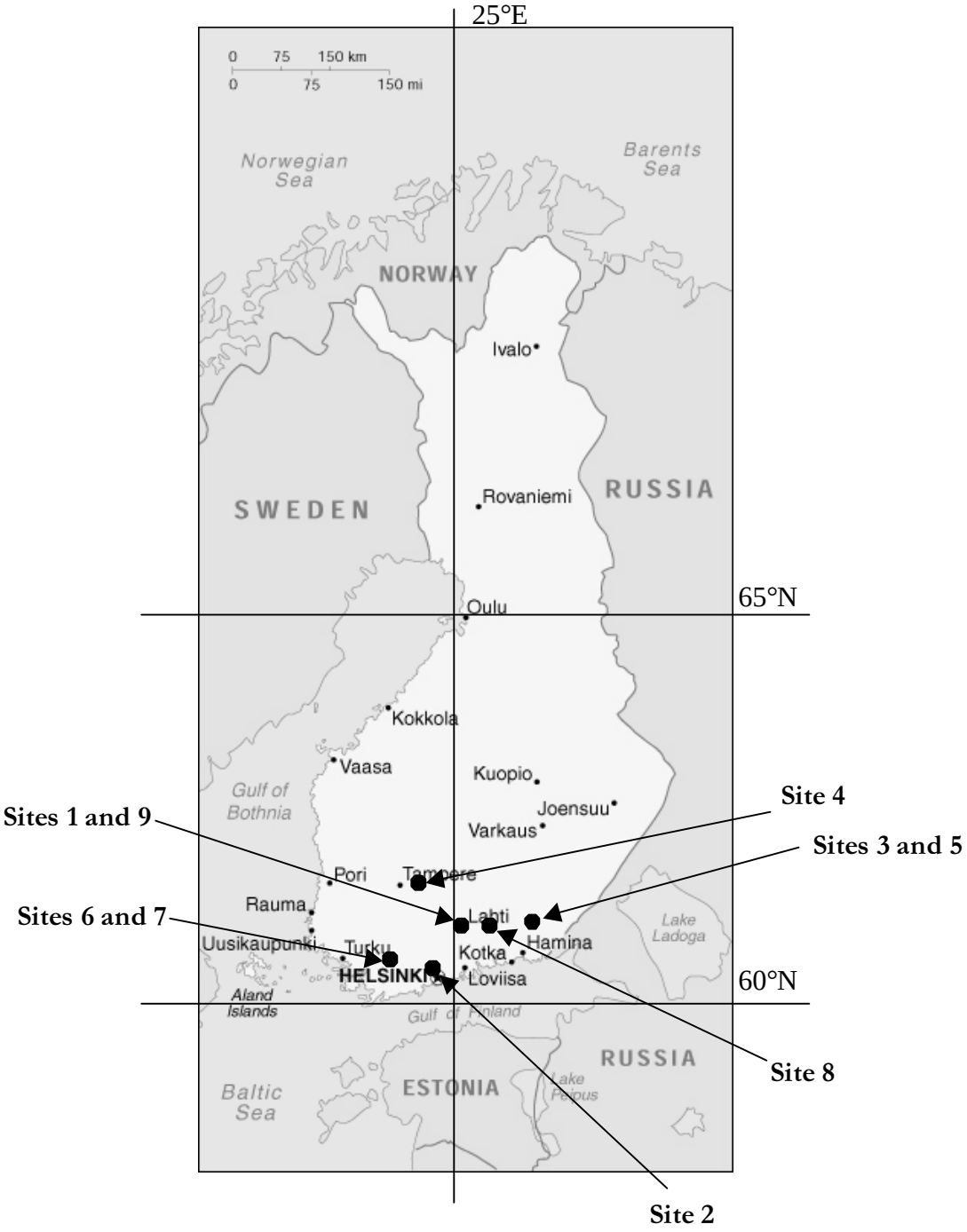

Fig. 3. Map of sampling sites 1-9.

Table 2. Sampling sites of this study.

SITE	DESCRIPTION [location, in operation (years)]	CONTAMINANTS (acid-soluble metals)	PAPER
1	Southern-Finland (61°N;25°E), 1956-1965	10-26100 mg kg^{-1} As 32-18500 mg kg^{-1} Cr 26-7000 mg kg^{-1} Cu	I, II
2	Southern-Finland (60°N; 25°E), 1980 →	3-100 mg kg^{-1} As	II
3	South-Eastern Finland (61°N;27°E), 1974 →	310-4600 mg kg^{-1} As, Cr, Cu	III
4	Southern-Finland (62°N;24°E), 1957-1968	760-92000 mg kg^{-1} As, Cr, Cu	III
5	South-Eastern Finland (61°N;27°E), 1945-1968	170-20100 mg kg^{-1} As, Cr, Cu	III
6	Southern-Finland (60°N;23°E), 1950-1980	100-340 mg kg^{-1} As 115-620 mg kg^{-1} Cr 30-310 mg kg^{-1} Cu	IV
7	Southern-Finland (60°N;23°E), 1975-1998	1270-3660 mg kg^{-1} As 430-1720 mg kg^{-1} Cr 460-1470 mg kg^{-1} Cu	IV
8	Southern-Finland (61°N;26°E) 1950-1980	900-8500 mg kg^{-1} As 600-6200 mg kg^{-1} Cr 210-2500 mg kg^{-1} Cu	IV
9	Southern-Finland (61°N;25°E) 1964-1987	9800 mg kg^{-1} Pb (surface soil) 330 mg kg^{-1} Pb (mineral soil)	V

3.2. Speciation and mobility of arsenic

Speciation studies of water soluble and gaseous arsenic forms and mobility studies of arsenic were carried out as microcosm experiments (Papers I, II). To determine if the microbial activity had an influence on the speciation and mobility of arsenic, formaldehyde (0.04 %) was added to "killed" control samples before incubation to inhibit microbial activity. The advantage of formaldehyde for inhibiting microbial activity is that it does not cause any changes in dissolved nutrient concentrations or pH in soil (Tuominen et al., 1994; Kairesalo et al., 1995). To determine in detail if the mobilization of arsenic was enhanced as a result of addition of a carbon source, different concentrations of glucose (0, 0.5 or 1.0 %) were added to soil microcosms.

The speciation analyses of water-soluble arsenic species [As(V), As(III), MMAA, DMAA] were carried out by ion chromatography-inductively coupled plasma mass spectrometry

(IC–ICP-MS) and the acid-soluble and the total water soluble cocentrations of arsenic were determined by hydride generation atomic absorption spectrometry (HG-AAS) or by ICP-MS. The volatile arsenic species were analyzed by gas chromatography-mass spectrometry (GC-MS). The uncertainties of measurements were: 5 % for ICP-MS, 10 % for IC–ICP-MS and 10 % for HG-AAS.

3.3. Bioavailability measurements

The aim of these experiments was to test a previously developed As(III)–specific bacterial sensor, *Escherichia coli* MC1061(pTOO31) (Tauriainen et al., 1999), for its applicability for analysis of As(V) bioavailability in contaminated soil samples (Paper III). M9 minimal medium (Sambrook et al., 1989) supplemented with 0.1% acid hydrolysed casein or LB-medium (Sambrook et al., 1989) was used for rehydration of lyophilised bacteria and for the incubation of the bacteria with samples. The bacteria were incubated together with the standards or samples (i.e. soil water extractions and soil suspensions) for 120 min at 37 ºC, after which 100 µl of 0.5 mM D-luciferin substrate (in 0.1 M citrate buffer) was added. The reaction mixture was incubated at room temperature for 15 min before measurement of luminescence. The measurement was performed with a luminometer.

In this method, the sensing of arsenic is based on controlling the expression of a reporter gene, firefly luciferase (*lucFF*), by the regulatory unit of the *ars* operon of *Staphylococcus aureus* plasmid pR773 in recombinant plasmid pTOO31, with *E.coli* MC1061 as the host strain (Tauriainen et al., 1999). The regulatory unit of the *ars* operon consists of the ars promoter and the repressor protein, ArsR. In the absence of arsenic, the expression of *lucFF* is repressed while in the presence of arsenic, transcription of the promoter is induced, and luciferase is produced at a level corresponding to the concentration of arsenic.

3.4. Microbial activity and community structure measurements

Wood impregnation has contaminated numerous sites due to the use of chromated copper arsenate (CCA) for timber treatment. In addition to arsenic also chromium (Cr) and copper (Cu) can have negative effects on the soil microbial activity in these areas. In this study, the effects of arsenic, chromium and copper contamination on the soil microbial community structure, potential microbial activity and arsenic-resistance were investigated at three field sites (Paper IV). Microbial community structure was determined by analysis of phospholipid fatty acids (PLFAs) and 16S rRNA gene terminal restriction fragment length polymorphism (t-RFLPs). Potential microbial activity was estimated by measuring $^{14}CO_2$ evolution from ^{14}C-glucose. Arsenic-resistant bacteria were enumerated on agar plates. A total of 90 randomly selected resistant colonies from As(III)-plates were tentatively identified by their fatty acid methyl ester profiles (FAME). The concentrations of soil acid-soluble and total water soluble arsenic, chromium and copper and the concentration of bioavailable arsenic (analyzed with a bacterial sensor) were also determined.

3.5. Phytostabilization of lead

A total of 24 soil microcosms were set up in the laboratory (Paper V). For measuring drainage, 20 holes were drilled through the bottom of each container. For the sampling of soil, 4 holes were drilled through the wall of each container. Into each microcosm, 1.5 kg of lead-contaminated or uncontaminated soil was added, as a 15 cm thick mineral soil layer on the bottom and a 5 cm thick organic soil layer on the top. The microcosms were incubated under controlled temperature and light conditions for 77 days. Into half of the microcosm of both soil types, a 20 cm high pine seedling was planted and $CaCO_3$ was added also to half of the microcosms. Each treatment had three replicates. The soil of the microcosm was sampled 5 times. Soil samples (to measure water soluble lead, bioavailable lead, DOC) were taken separately from surface and mineral soils. Samples for plant roots, stems and

needles were taken at the end of the experiment. For analyzing the mobility of lead, the soil moisture was raised to 55 % at day 55 and the leachate was collected into plastic containers.

3.6. Phytostabilization of arsenic

The soil types, treatments and measurements of phytostabilization experiment with arsenic contaminated soil are presented in Table 3. The experiment was carried out in microcosms, as described in section 3.5.

Table 3. The soil types, treatments and measurements of phytostabilization experiment with arsenic contaminated soil.

SOIL (acid soluble As concentration mg kg^{-1})	**TREATMENT**	**MEASUREMENTS (sampling days)**
Control (As concentration < 10 mg kg^{-1})	**no plant**	**acid soluble As** (Day 1)
	Pinus sylvestris	**water soluble As** (Day 1, 14, 28, 42, 56)
As-I (5000 mg kg^{-1} As)	***Betula pendula***	**speciation of As** (Day 1, 28, 56)
	Festuca ovina	**bioavailable As (using a biosensor)** (Day 1, 14, 28, 42, 56)
As-II (15000 mg kg^{-1} As)		**As concentration in drainage** (Day 56)
		As concentration in plants* (Day 56)
		activity of plants** (transport of ^{14}C-labeled photosynthetic products of plants to the roots) (Day 56)

***** The concentration of arsenic in plants was measured separately from plant leaves, stems and roots, exept in *Festuca ovina*, in which the leaves included both stem and leaves (i.e. the shoot). Homogenized and dried plant samples were digested by wet oxidation (HNO_3; autoclave for 30 min). After digestion, the samples were diluted with water and the arsenic concentration was analyzed with ICP-MS.

****** The activity of plants was measured as the accumulation of ^{14}C-labeled photosynthetic products of plants to the leaves and stems, and as the transport of these products to the plant roots. For the measurement, plants (*Pinus sylvestris; Betula pendula; Festuca ovina*) were incubated with ^{14}C-CO_2 for 8 hours in light and 16 hours in darkness. After incubation, samples from leaves, stems and roots of plants were collected and combusted in a Junitek Oxidizer (Turku, Finland). The evolved $^{14}CO_2$ was trapped into Lumasorb II and analyzed with a liquid scintillation counter (Wallac 1414 Win Spectral™).

3.7. Methods used in this study

The analytical methods used in this thesis are listed in Table 4. References to the published methods and modifications made to them are described in each article.

Table 4. Methods used in this study.

Method	Paper number
- Analysis of soil acid soluble As concentration (8 M HNO_3 extraction in autoclave for 30 min)	I, II, III, IV
- Modified Tessier´s fractioning of soil samples for As analysis	I
- Speciation analysis of water soluble As forms (IC–ICP-MS)	I, II
- Toxicity test, BioTox™	I
- Analysis of water soluble As concentration (shaking for 60 min with dH_2O)	II, III, IV
- Speciation analysis of gaseous As forms (GC-MS)	II
- Speciation analysis of As(III) and As(V), cartridge method	III, IV
- Analysis of bioavailable As(V), *Escherichia coli* MC1061(pTOO31)	III, IV
- Soil PLFA	IV
- 16S rRNA gene t-RFLP	IV
- ^{14}C-glucose mineralization	IV
- Enumeration of culturable As-resistant bacteria, CFU	I, IV
- Identification of bacterial strains, FAME	IV
- Analysis of soil acid-soluble and water soluble Cr and Cu (8 M HNO_3 extraction in autoclave for 30 min; shaking for 60 min with dH_2O)	IV
- Dehydrogenase activity	V
- Analysis of acid-soluble and water soluble Pb (8 M HNO_3 extraction in autoclave for 30 min; shaking for 60 min with dH_2O)	V
- Analysis of bioavailable Pb, *Staphylococcus aureus* RN4220(pTOO24)	V
- Analysis of plant Pb concentration (wet oxidation with HNO_3)	V
- Analysis of plant As concentration (wet oxidation with HNO_3)	This thesis
- Activity of plants (transport of photosynthetic products of plants to the roots)	This thesis

4. RESULTS AND DISCUSSION

4.1. Speciation and mobility of arsenic in soil

The influence of microbes on the speciation of arsenic was studied under laboratory conditions (Papers I, II). In the beginning of the experiments, almost all (> 99.9%) of the arsenic existed as As(V). During the laboratory incubations (5 or 10 days) some methylation and reduction took place. The degree of species conversion was, however, not very high, being < 0.5 % compared to the original As(V) concentration (Paper I, Tables 4 and 5). Also, according to Cullen and Reimer (1989), Masscheleyn et al. (1991), Pongratz (1998) and Balasoiu (2001) the most often encountered arsenic form in soils is As(V). It is, however, possible that more reduction of As(V) to As(III) occurs in soils. If As(III) is rapidly oxidized, either microbiologically or chemically, back to As(V), only low concentrations of As(III) can be detected. The conversion of arsenic species during the extraction and storage of soil samples have been studied by Pantsar-Kallio and Manninen (1997). The authors reported that no conversions of As(V) to As(III) was detected and also that the recoveries of As(III) were near 100 % at pH-values 3-9. Thus, the reason for only low concentration of As(III) was not its rapid oxidation during the extraction and storage of soil samples.

It is known that inorganic As(V) is subject to microbial reduction and methylation leading to volatilization as arsines (Alexander, 1977; Woolson, 1977a; Cheng and Focht, 1979; Gao and Burau, 1997). However, the reduction and/or methylation rates of arsenic, necessary pre-requisities to production of arsine, MMA, DMA and TMA, vary greatly depending on soil properties such as soil pH (Carbonell-Barrachina et al., 1999) and soil moisture, temperature, abundance of different species of arsenic and microbial populations in soil (Gao and Burau, 1997). In our study soils, the microbial transformation rate of water soluble As(V) under both aerobic and anaerobic conditions to volatile TMA represented 0.02-0.3 % (Paper II, Fig. 6). The production of TMA in soils was lower in this study when compared to previous studies; for example Atkins and Lewis (1976), Woolson (1977b) and Woolson and Kearney (1973) observed that 11 %, 18 % and 61 %, respectively, of soil acid-soluble arsenic concentration was converted to TMA. However, Gao and Burau (1997) found that there might be large variation in the transformation rates of arsenic. According to their results, the demethylation of arsenic was much higher (3-87 % of soil acid-soluble arsenic concentration) than the loss of arsenic as arsines (0.001-0.4 %). This indicates that the loss of arsenic from some soils to the atmosphere may not be an important pathway and inorganic arsenic may accumulate in some soils.

TMA was detected also from the landfill of arsenic contaminated soils (Paper II, Fig. 4). Even though the concentration of soil acid-soluble arsenic was an order of magnitude lower in the field than in soil used in laboratory experiments, the concentration of TMA produced under field conditions was higher than under laboratory conditions. In the field, the gas samples were, however, taken from the soil gas phase, not diffused out from the soil, like in the laboratory experiment. This indicates that gaseous arsines may concentrate inside the soil and are emitted from the soil to the atmosphere mainly when treating, e.g. turning over, the soil material. According to Pantsar-Kallio and Korpela (2000) the stability of the gaseous arsenic species can be different in those soil sites where they are formed compared with their stability in surrounding air. Thus, the distance arsines may travel in air depends on environmental conditions (Pongratz, 1998). The stability of TMA is, however, higher than the stability of arsine (AsH_3) and therefore TMA is the most often encountered gaseous arsenic species in the environment (Pantsar-Kallio and Korpela, 2000).

Even though the microbial transformation rates of As(V) were low, microbes were able to enhance the leaching of arsenic into the soil water (Paper I, Fig. 1). Also Ahmann et al. (1997) and Bachofen et al. (1995) have reported that in autoclaved, filtered or

formaldehyde-killed samples the leaching of arsenic was much lower than in "live" samples. In our study, most of the arsenic was leached as As(V). However, arsenic can be mobilized also through microbial reduction of As(V) to As(III). As(III) is more mobile than As(V), and thus the microbially mediated species conversion may enhance the mobilization of arsenic. For example, Macur et al. (2001) reported that microbial reduction of As(V) to As(III) in arsenic-contaminated soils may occur relatively fast resulting in enhanced arsenic mobilization. Macur et al. (2001) found also, that reduction of arsenic occurred under aerobic conditions, thus indicating that the reduction of As(V) was not coupled to anaerobic respiration, but was more likely a detoxification mechanism. However, also dissimilatory As(V)-reducing bacteria have been implicated in the mobilization of arsenic (Niggemyer et al., 2001). Also, various field experiments have demonstrated that arsenic may be mobilized and transported from contaminated soils to groundwaters or through brooks and rivers to surrounding lakes (Räisänen et al., 1997; Kalbitz and Wennrich, 1998; Lyytikäinen et al., 2001; Cai et al., 2002).

In this study, however, microbes needed a carbon source (glucose addition) before they functioned as effective bioleachers (Paper I). Also, McCreadie et al. (2000) and Balasoiu et al. (2001) found, that addition of organic carbon may stimulate bacteria, which mobilize arsenic. However, according to authors, the mobilization was due to microbial reduction of As(V) to more mobile As(III). In this study, arsenic was mobilized also in "killed" samples. Thus, physicochemical leaching in addition to biological leaching was a significant mechanism for mobilizing arsenic from soil. In this case phosphate, carbonate and chloride anions in minimum medium solution could have leached arsenic anions from the soil by ion exchange mechanism (Pantsar-Kallio and Manninen 1997).

4.2. Bioavailability of As(V) in contaminated soils

The bioavailable arsenic content of contaminated soils was determined by joint analyses of acid-soluble, total water soluble and bioavailable arsenic using a luminescent bacterial sensor *Escherichia coli* MC1061(pTOO31) (Paper III). The dominant (> 99 %) arsenic species in contaminated soils studied was As(V). Therefore, a previously developed As(III)–specific bacterial sensor, *Escherichia coli* MC1061(pTOO31) (Tauriainen et al., 1999), was tested for its applicability for analysis of As(V) contaminated soil samples. In M9 medium the bacteria reacted to As(III) at 100 times lower concentrations compared to As(V). In nutrient-rich LB-medium, however, the difference in the concentrations causing the response was significantly smaller. The sensitivity of the test for As(III) was approximately the same in both media (about 7.5 $\mu g\ l^{-1}$, 0.1 μM), but for As(V) the sensitivity improved from ~750 $\mu g\ l^{-1}$ (10 μM) to ~ 35-40 $\mu g\ l^{-1}$ (0.5 μM), i.e. approximately 20-fold in LB-medium (Paper III, Fig. 2). Therefore, LB-medium was used to rehydrate the bacterial biosensors in this study [Paper III, Fig. 3 (Standards), Fig. 4 (Water extractions), Fig. 5 (Soil suspensions)]. According to Diorio et al. (1995) the improved sensitivity for As(V) in LB-medium may be due to the higher expression of chromosomal arsenate reductase of *E. coli*. It is also possible that the rich medium provides a more suitable environment for biological reduction of As(V) by the reductase enzyme.

In contaminated soils, a significant correlation was found between the concentration of total water soluble arsenic and the bioavailability of arsenic (Paper III, Fig. 4). The bioavailability of arsenic, however, varied between sampling sites and was not predictable from the soil acid-soluble arsenic concentration or from the soil water soluble arsenic concentration; bioavailable arsenic was 3-77 % of total water soluble arsenic in soil. This indicates that the acid-soluble arsenic concentration is not a good indicator of arsenic bioavailability and apparent toxicity. Also, even if the water soluble arsenic content is useful in risk assessment of arsenic leaching to the ground water (Wenzel et al., 2001), it does not provide a relevant prediction of the bioavailability of arsenic in soils.

The bioavailability of arsenic was highly site-specific (Paper III, Table 1) indicating that many factors may affect the bioavailability of metals in soil. Similar results were reported in the study of Bååth (1989), who found that soil clay content decreased the bioavailability of metals. In addition, according to Barkay et al. (1997) dissolved organic carbon decreased the bioavailability of mercury. However, Giller et al. (1998) reported, that decreased pH resulted in increased bioavailability of metals, such as zinc, nickel and cadmium, in soil. In this study, the bioavailability of arsenic was considerably lower (15 % of water soluble arsenic) at old and abandoned sampling sites (in operation before 1968) than at a site, which was still in use (35 % of water soluble arsenic) during the studies (year 2000). This suggests that the arsenic-compounds that have aged in the soil are less bioavailable than when freshly added to soil. According to Alexander (2000) and Lock and Janssen (2001) aging of zinc, cadmium, nickel and cobolt has also been shown to occur in contaminated soils. This declining bioavailability (i.e. the bioavailability of arsenic may decline with little or no reduction in the acid-soluble arsenic concentration) is not, however, reflected by currently used methods for risk assessment of arsenic contaminated soils (Khan and Scullion, 2000; Chew et al., 2001; Kunito et al., 2001). As a consequence, the risks of abandoned and old contaminated soils may often be overestimated, which might lead to wrong decisions in choosing arsenic contaminated sites for remediation.

Bacterial biosensors can be used to give information on the bioavailability of arsenic in soils (Paper III). However, the toxicity and bioavailability of arsenic differs between organisms (Plette et al., 1999) and the results from bacterial sensor tests can not be applied directly to the higher organisms, such as plants and human beings. Another difficulty of using bacterial sensors is that the biological component is a viable cell, and therefore bioavailability measurements are limited to conditions which allow survival of the cell, e.g. narrow pH and temperature ranges (Bontidean et al., 2000). Also, because the promoter used is specific to arsenic, this technique is not well suited for universal “blind toxicity” screening. However, the use of bacterial biosensors is simple, rapid and inexpensive and therefore, this technique is potentially an ideal tool for preliminary screening of contaminated soils.

4.3. Microbial activity and community structure in arsenic, chromium and copper contaminated soils

The effects of arsenic, chromium and copper contamination on the microbial community structure and potential microbial activity were investigated in soils of old and abandoned wood impregnating plants (Paper IV). The microbial community structure analyses, PLFA and t-RFLP, indicated that exposure to high metal contamination or subsequent effects (for example lower plant growth) of this exposure can permanently change the microbial community strucure (Paper IV, Figs. 5 and 7). Specifically, in PLFA analyses, a decrease in several iso- and anteiso-branched PLFAs, all commonly found in gram-positive bacteria, was found in highly contaminated soils. Evidence for a similar shift was found in studies reported by Jordan and Lechevalier (1975), Doelman and Haanstra (1979) and Hiroki (1992). A further indication that such sift had occurred in our study was indicated by the increase in cy17:0, which is consireded to be typical for gram-negative bacteria (Lechevalier, 1977). Also, an increase in 18:2ω6,9, an indicator fatty acid for fungi was found at highly contaminated sites. Similar results were found in the studies of Jordan and Lechevalier (1975), Hiroki (1992) and Khan and Scullion (2000). However, several methyl-branched fatty acids, which are typical for actinomycetes, responded differently to metal contamination. This indicates that different members of the actinomycete population can respond differently to elevated metal concentrations, which was found also in a study of Kelly et al. (1999).

The t-RFLP results (Paper IV, Fig. 6) indicated, that the microbial community is able to compensate for the reduced population size and diversity with time. According to t-RFLP results, this was not due to a reversion towards the pre-exposure community but mainly due to the appearance of new dominating species. Similar results were reported by Maliszewska et al. (1985) who found that As(V) stimulated the proliferation of certain groups of microorganisms in soil resulting in a shift of the microbial community comprise only a few tolerant species. Also, Rasmussen and Sorensen (2001) found that microbial diversity decreased immediately after exposure to mecury but also that adaptation to mercury stress may result in a recovery of diversity due to a shift in the community structure.

Even though the microbial community structure was altered in higly contaminated soils, no differences were noted in glucose mineralization ($^{14}CO_2$ evolution from ^{14}C-glucose) or in the total number of heterotrophic colony forming units (CFU) among contaminated and control soil samples within sites (Paper IV, Fig. 1 and Table 3). This indicates that the microbial populations at contaminated sites were well adapted even to high concentrations of arsenic, chromium and copper. Also Pennanen et al. (1996) reported that at long-term field sites, soil microbial communities have had time to adapt to the stress presented by the elevated metal concentrations. In addition, the results were supported by the finding of Konopka et al. (1999) that the microbial metabolic potential of readily degradable substrates, such as glucose, was not inhibited by lead, even though high concentrations of lead changed the soil microbial community structure. Similar results were found in the studies of Dahlin et al. (1997) and Bååth et al. (1998b) who reported that significant differences were found in the microbial community structure determined by PLFA patterns between heavy metal treated soils and control soils. According to authors, the microbial activity or basal respiration was not, however, affected by metal additions.

Arsenic can be classified as a potentially hazardous metal at old wood impregnating plants (Hingston et al., 2001; Hingston et al., 2002), because of high long-term solubility of arsenic compared to chromium and copper (McLaren et al., 1998; Ruokolainen et al., 2000). This was found also in soil of this study; the solubility of arsenic was 10-200 fold higher than the solubility of chromium or copper. Therefore, special attention was paid to the examination of arsenic-resistance and characterization of the resistant community members. In the contaminated soils studied, the proportion of As(V)-resistant bacteria was high and relatively independent of the soil arsenic concentration (Paper IV, Table 3). In contrast, the proportion of As(III)-resistant bacteria was low and dependent on the concentration of arsenic in the soils. This indicates that As(III) is more toxic to culturable microbes than As(V), which was also found in study of Maliszewska et al. (1985).

Characterization and identification of the arsenic-resistant bacterial isolates from three study sites supported the conclusion based on PLFA and t-RFLP microbial community analyses, which revealed considerable differences between the three sampling sites. According to FAME analyses, arsenic-resistant genera included *Salmonella, Pseudomonas, Edwardsiella, Enterobacter, Acinetobacter* and *Serratia* species (Paper IV, Fig. 3). The isolated strains showed a relatively high resistance against As(III) and As(V), and among them the *Acinetobacter radioresistens, A. baumannii, Pseudomonas chlororaphis and P. syringae* were extremely resistant. The dominant arsenic-resistant organisms found at the most contaminated site were *A. radioresistens and A. baumannii* (Paper IV, Fig. 3). One of the main peaks found in the t-RFLP pattern corresponded to the *Acinetobacter* species. This suggests that the culturable organisms may be important members of microbial communities in soils. Also, Nieto et al. (1989) and Deshpande et al. (1993) have found that *Acinetobacter* species are highly resistant to metals and that several *Acinetobacter* sp. strains are commonly found at metal contaminated sites. The results of Macnaughton et al. (1999), that the major changes in the microbial community structure of metal-treated

microcosms consisted of the appearance of *Acinetobacter* sp. strains, correspond to the results of this study.

The identification of arsenic-resistant isolates was, however, tentative, because it was based only on one method (FAME analysis). For a more reliable identification of microbial species, it is recommended to use two or three different methods, such as FAME and 16S rDNA sequencing, combined.

In summary, this study indicates that microbes are able to respond to soil metal contamination and maintain the metabolic activity apparently through changes in microbial community structure and selection for resistance. The changes in microbial community structure and diversity were site-specific and were not related to the soil acid-soluble metal concentration. Thus, the differing findings in microbial community structure in metal contaminated soils may have resulted from variations in the levels of metal bioavailability. Similar results were found in the study of Rasmussen et al. (2000) who reported that the microbial response to mercury was observed as development of mercury-resistant bacteria and lowering of diversity, which correlated with changes in concentrations of bioavailable mercury.

4.4. Phytostabilization of lead

Effects of pine (*Pinus sylvestris*) and liming (pH-change with $CaCO_3$) on the solubility, mobility and bioavailability of lead in boreal forest soil, previously used as a shooting range area, were examined in laboratory microcosms (Paper V). Pine seedlings had a major role in immobilization of lead in contaminated soil. In boreal forest soil, the reduction of water soluble lead was 0-56 % in humic rich surface soil and 12-93 % in mineral soil (Paper V, Fig. 2). In the surface and mineral soil the reduction in mobility was 40-57 % (Paper V, Table 2). These results showed that pine seedlings had a major role in the immobilization of lead in the contaminated soil. Similar results were reported by Berti and Cunningham (1997) who found that in-place inactivation, i.e. the use of green plants to remediate lead contaminated soils, reduces the hazards associated with lead contaminated soils through chemical and physical stabilization. Also, Cotter-Howells and Caporn (1996) found that the roots of *Agrostis capillaris*, growing in highly contaminated lead/zinc mine wastes, caused the formation of pyromorphite, a highly unsoluble and non-bioavailable lead phosphate mineral, thus decreasing the solubility of lead in contaminated soils. Lead stabilization is soil specific and depends on the level of lead contamination and soil characteristics controlling the solubility and mobility of lead, and more likely the phytostablization of lead may be efficient remediation method in soils with relatively low levels of contamination (Klassen et al., 2000).

Sauve et al. (1997) reported that the relation of dissolved lead and Pb^{2+} in the soil solutions of various contaminated soils can be predicted from simple soil properties like pH, soil total lead and phospate concentration. Various sequential extraction techniques have been used to predict the bioavailability of soil metals (Tessier, 1979; Krisnamurti et al., 1995; Gupta et al., 1996; Morera et al., 2001). Biosensors, however, provide a direct and more accurate determination of metal bioavailability. In this study, the bioavailability of lead was assessed directly using a lead-specific luminescent bacterial sensor, *Staphylococcus aureus* RN4220(pTOO24), in which the lead resistance promoter controls the expression of firefly luciferase. Significant positive correlation was found between the concentration of total water soluble lead and the bioavailability of lead in the soils (Paper V, Fig. 4). The concentration of bioavailable lead was not, however, predictable from the concentration of total water soluble lead; bioavailable lead was only 4-6 % of total water soluble lead in humic surface soil and 13-43 % in mineral soil (Paper V, Table 4). This is of great importance because ionic species (Pb^{2+}) are mainly responsible for the biological effect of lead in soils (Sauve, 1999). Thus, the lead bioavailability measurements indicate low

toxicity of lead to soil microbes even when the soil total lead concentration is high. The results of dehydrogenase activity measurements of highly lead-contaminated boreal forest soil supported this result. In lead-contaminated soils the microbial activity was reduced only slightly when compared to uncontaminated control soils (Paper V, unpublished data, Fig. 4).

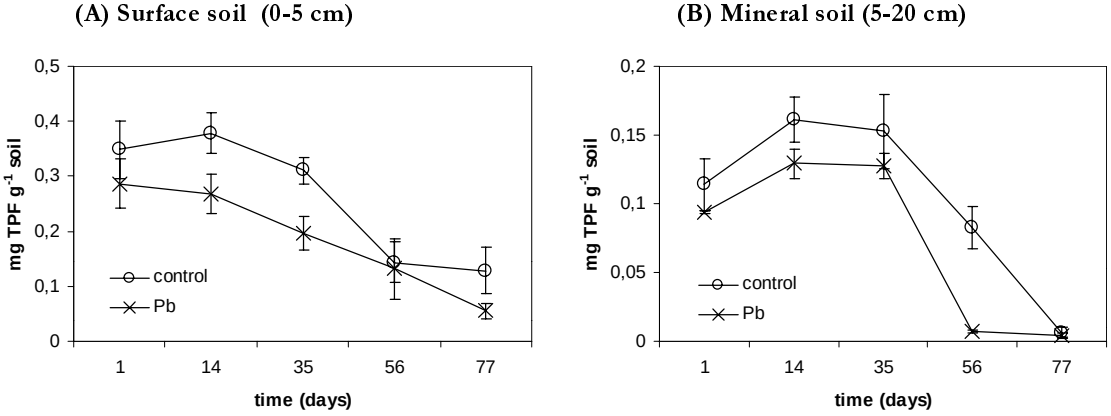

Fig. 4. Microbial dehydrogenase activity (mg TPF g^{-1} soil) in Pb-contaminated (Pb) and uncontaminated (control) (A) surface soil and (B) mineral soil during 77-day laboratory experiment (mean ± SD, n =3).

In acidic soils with high total lead concentrations, the solubility and bioavilability of lead is often elevated (Wang and Benoit, 1996; Fang and Wong, 1999). Thus, liming (increasing pH) should decrease the solubility, bioavailability and toxicity of lead in soils (Derome, 2000). However, according to results of this study, liming (pH increase from 3.5-4.3 to 6.5) did not reduce the solubility, mobility or bioavailability of lead in the soil (Paper V, Fig. 2 and Table 2), indicating that soil pH is not the only factor affecting mobility and bioavailability of lead in boreal forest soils. One possible reason for the increased solubility and mobility of lead can be the higher formation of dissolved organic lead-complexes as a result of elevated soil pH (Reddy et al., 1995; Sauve et al., 1998). In addition, Harter (1979) reported that the relationship between pH and lead adsorption in soil is not straightforward and thus he suggested that total bases would give a better prediction of lead adsoprtion in soils because it is a function of both pH and cation exhange capacity. According to Harter (1979), the high amount of calsium ions could have resulted in increased ion exchange of Pb^{2+} and resulted in elevated concentrations of soluble and bioavailable lead.

4.5. Phytostabilization of arsenic

Because the results of phytostabilization experiments with lead contaminated soils were promising, the same method was tested for arsenic contaminated soils. However, in arsenic contaminated soils, the tested plants (*Pinus sylvestris, Betula pendula*, *Festuca ovina*) did not decrease the solubility, mobility or bioavailability of arsenic in soil (unpublished, Fig. 5). Arsenic was highly toxic to plants and decreased the activity of plant roots (i.e. the transport of ^{14}C-labeled photosynthetic products of plants to the roots) (unpublished, Fig. 6), which could be one reason for the low phytostabilization potential of the plants tested. However, the plants decreased the species conversion of As(V) to As(III) in deeper soil layer (15 cm) (unpublished, Fig. 5), and thus the presence of plants may have an important role in detoxification of arsenic. Similar results were found in the study of Otte et al. (1992) who reported that due to oxidizing activity of plant roots and/or microorganisms, As(III) may be oxidized to As(V) in the rhizosphere of plants. In addition, *Festuca ovina* accumulated high concentrations of arsenic into its roots and shoots (unpublished, Table 5), and thus this grass could be used for phytoextraction of arsenic contaminated surface soils. Ma et al. (2001) and Visoottiviseth et al. (2002) have also found that for example *Pteris*

vittata, Pityrogramma calomelanos, Mimosa pudica and Melastoma malabrathricum accumulate high concentrations of arsenic, and therefore these plants seem to be suitable for phytoextraction.

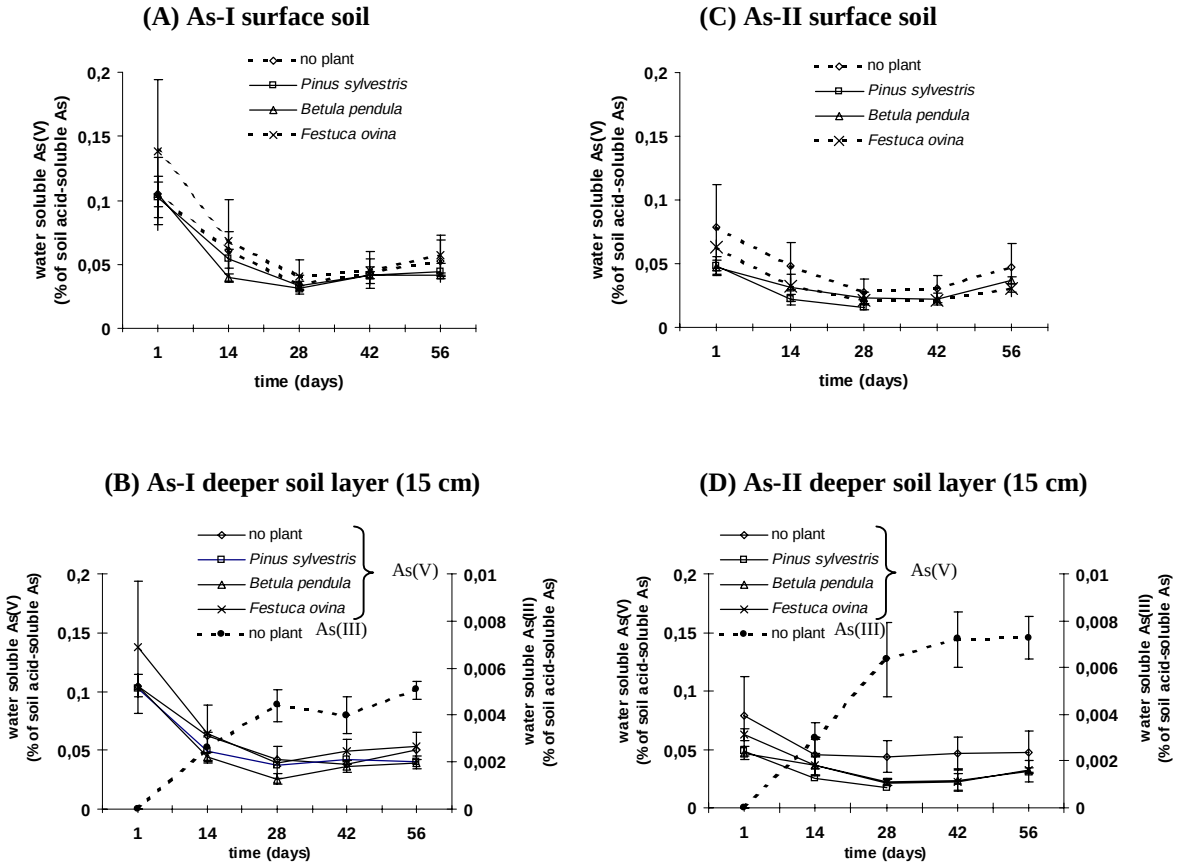

Fig. 5. Water soluble As(V) and As(III) (as percentage of soil acid-soluble arsenic) during 56-day laboratory experiment with no plant; with *Pinus sylvestris*; with *Betula pendula*; and with *Festuca ovina*. (A) As-I (5000 mg As kg^{-1}) surface soil; (B) As-I (5000 mg As kg^{-1}) deeper soil layer; (C) As-II surface soil (15000 mg As kg^{-1}); and (D) As-II (15000 mg As kg^{-1}) deeper soil layer.

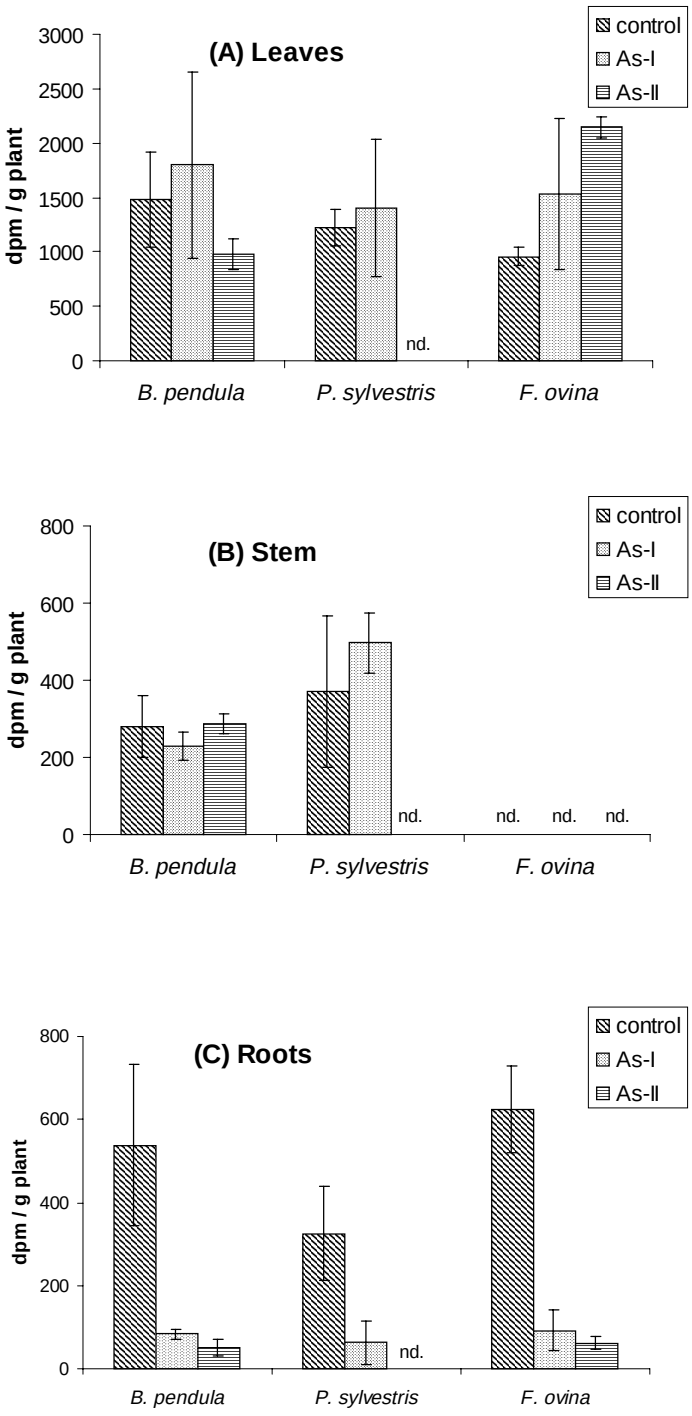

Fig. 6. Accumulation of ^{14}C-labeled photosynthetic products of plants (*Betula pendula, Pinus sylvestris* and *Festuca ovina*) in (A) leaves; and (B) stems, and their transport to (C) roots after 56 days of laboratory incubation in uncontaminated (control); As-I (5000 mg As kg^{-1}); and As-II (15000 mg As kg^{-1}) soils (mean $\pm$ SD, n =3).

Table 5. Concentration of arsenic (mg As kg^{-1}) in roots, stem and leaves of *Pinus sylvestris, Betula pendula* and *Festuca ovina* after 56 days of laboratory incubation in As-I (5000 mg As kg^{-1}) and As-II (15000 mg As kg^{-1}) soils (mean ± SD, n =3). In uncontaminated (control) soils, the concentration of arsenic in roots, stem and leaves of plants was below 3 mg kg^{-1}.

	Mg As kg^{-1}		
As-I	**Roots**	**Stem**	**Leaves**
Pinus sylvestris	773±286	42±7.2	76±14
Betula pendula	353±50	< 3	11±1.2
Festuca ovina	863±293	nd.	1367±116
As-II			
Pinus sylvestris	433±133	330±120	353±110
Betula pendula	340±111	11±2.3	11±2.3
Festuca ovina	2533±551	nd.	3200±700

5. CONCLUSIONS – ENVIRONMENTAL SIGNIFICANCE

In order to be able to exploit the potential of bioremediation and phytoremediation, basic environmental phenomena like the effects of microbes and plants on metals have to be understood. This thesis gives insight how these phenomena could be exploited when developing bioremediation methods for metal, especially arsenic and lead, contaminated soils.

Unlike organic pollutants, that can be mineralized to harmless products such as CO_2, arsenic and lead cannot be biodegraded, but persist indefinitely, complicating the remediation of contaminated soils. Therefore, the main strategy is to reduce the bioavailability, mobility and toxicity of the metal. Biological methods for remediation of arsenic- and lead-contaminated soils include detoxification, bioleaching and phytoremediation. In arsenic-contaminated soils, microbial methylation of inorganic arsenic to water soluble methylated arsenic forms, MMAA and DMAA, may function as a detoxification method. However, in soils tested in this study, biomethylation of arsenic was of minor importance. Also, the possibility that MMAA and DMAA, may be transformed to highly toxic volatile arsines by biomethylation has to be taken into account in the bioremediation of arsenic-contaminated soils.

The bioavailability of arsenic was low at all sites when compared to the acid-soluble arsenic concentration. This can explain why biomethylation was not a common microbial transformation process in the soils tested. Even though the results of this study revealed that biomethylation activity in arsenic-contaminated soils was low, arsenic was bioleached [mainly as As(V)] in laboratory microcosms. Therefore, as a result of enhanced microbial activity due to addition of nutrient rich or organic soil, the mobilization of arsenic from soil may be increased and result in pollution of groundwaters and downstream lakes. Thus, bioleaching is not a suitable *in situ* bioremediation method. More likely, bioleaching could be a potential *ex-situ* bioremediation method in which the contaminated soils are treated in closed tanks or reactor vessels.

The PLFA and t-RFLP profiles and microbial activity measurements indicated that the microbial community is able to adapt to arsenic, chromium and copper contamination and maintain the metabolic activity through changes in the microbial community structure towards higher resistance for metals. The dominant arsenic-resistant bacteria in highly contaminated soils were *Acinetobacter radioresistens* and *A. baumannii* strains.

In lead-contaminated boreal forest soils tested in this study, the presence of pine seedlings significantly reduced the solubility, mobility and bioavailability of lead. This result indicates that the use of deep-rooted plants (such as pine) may serve as an appropriate remediation tool (through phytostabilization), at least decreasing the downward leaching of lead and thereby the risk to groundwater contamination. However, in arsenic-contaminated soils, plants (*Pinus sylvestris, Betula pendula, Festuca ovina*) did not decrease the solubility, mobility or bioavailability of arsenic. Arsenic was highly toxic to plants and inhibited the metabolism of plant roots, which could be one reason for low phytostabilization potential of plants tested. However, the plants decreased the formation of As(III) and thus the presence of plants may have an important role in detoxification of arsenic. Also, *Festuca ovina* accumulated high concentrations of arsenic into its roots and shoots, indicating that this grass could be employed for phytoextraction of arsenic-contaminated surface soils.

6. REFERENCES

Ahmann, D., Roberts, A.L., Krumholz, L.R., Morel, F.M.M. 1994. Microbes grow by reducing arsenic. *Nature* 371, 750.

Ahmann, D., Krumholz, L.R., Hemond, H.F., Lovley, D.R., Morel, F.M.M. 1997. Microbial mobilization of arsenic from sediments of the Aberjona watershed. *Environ Sci Technol* 31, 2923-2930.

Alexander, M. 1977. Introduction to soil microbiology. 2nd Edition. John Wiley&Sons, Inc. New York.

Alexander, M. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ Sci Technol* 34, 4259-4265.

Andrewes, P., Cullen, W., Polishchuk, E. 2000. Arsenic and antimony biomethylation by *Scopulariopsis brevicaulis*: Interaction of arsenic and antimony compounds. *Environ Sci Technol* 34, 2249-2253.

Atkins, M.B., Lewis, R.J. 1976. Chemical distribution and gaseous evolution of arsenic-74 added to soils as DSMA-^{74}As. *Soil Sci Soc Am J* 40, 655-658.

Bååth, E. 1989. Effects of heavy metals in soil on microbial processes and populations (a review). *Water Air and Soil Pollution* 47, 335-379.

Bååth, E., Arnebrandt, K., Nordgren, A. 1991. Microbial biomass and ATP in smelter-polluted forest humus. *Bull Environ Contam Toxicol* 47, 278-282.

Bååth, E., Frostegård, A., Fritze, H. 1992. Soil bacterial biomass, activity, phospholipid fatty acid pattern and pH tolerance in an area polluted with alkaline dust deposition. *Appl Environ Microbiol* 58, 4026-4031.

Bååth, E., Frostegård, Å., Montserrat, D-R., Tunlid, A. 1998a. Microbial community-based measurements to estimate heavy metal effects in soil. The use of phospholipid fatty acid patterns and bacterial community tolerance. *Ambio* 27, 58-61.

Bååth, E., Diaz-Ravina, M., Frostegård, A., Campbell, C.D. 1998b. Effect of metal-rich sludge amendements on the soil microbial community. *Appl Environ Microbiol* 64, 238-245.

Bachofen, R., Birch, L., Buchs, Ferloni, P., Flynn, I., Gaudenz, J., Tahedl, H., Chasteen, T.G. 1995. Volatilization of arsenic compounds by microorganisms. In: Hinche, R.E., Means, J. L., Burris, D. R. (Eds.) Bioremediation of inorganics, pp. 103-108.

Balasoiu, C., Zagury, G., Deshenes, L. 2001. Partitioning and speciation of chromium, copper and arsenic in CCA-contaminated soils: influence of soil composition. *Sci Tot Environ* 280, 239-255.

Bardgett, R.D., Saggar, S. 1994. Effect of heavy metal contamination on the short-term decomposition of labelled (^{14}C) in a pasture soil. *Soil Biol Biochem* 26, 727-733.

Barkay, T., Gillman, M., Turner, R.R. 1997. Effects of dissolved organic carbon and salinity on bioavailability of mercury. *Appl Environ Microbiol* 63, 4267-4271.

Barkay, T., Schaefer, J. 2001. Metal and radionuclide bioremediation: issues, considerations and potentials. *Current Opinion in Microbiology* 4, 318-323.

Berti, W.R., Cunningham, S.D. 1997. In-place inactivation of Lead in Lead-contaminated soils. *Environ Sci Technol* 31, 1359-1364.

Bindler, R., Bränvall, M-L., Renberg, I. 1999. Natural lead concentrations in pristine boreal forest soils and past pollution trends: A reference for critical load models. *Environ Sci Technol* 33, 3362-3367.

Blair, P.C., Thompson, M.B., Bechtold, M., Wilson, R.E., Moorman, Fowler, B.A. 1990. Evidence for oxidative damage to red blood cells in mice induced by arsine gas. *Toxicology* 63, 25-34.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I. 1997. Enhanced accumulation of lead in indian mustard by soil-applied chelating agents. *Environ Sci Technol* 31, 860-865.

Bontidean, I., Lloyd, J.R., Hobman, J.L., Wilson, J.R., Csöregi, E., Mattiasson, B., Brown, N.L. 2000. Bacterial metal-resistance proteins and their use in biosensors for the detection of bioavailable heavy metals. *J Inorg Biochem* 79, 225-229.

Brennan, M.A., Shelley, M.L. 1999. A model of the uptake, translocation and accumulation of lead (Lead) by maize for the purpose of phytoextraction. *Ecological Engineering* 12, 271-297.

Brim, H., McFarlan, S.C., Fredrickson, J.K., Minton, K.W., Zhai, M., Wackett, L.P., Daly, M.J. 2000. Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nature Biotechnology* 18, 85-90.

Bruins, M.R., Kapil, S., Oehme, F.W. 2000. Microbial resistance to metals in the environment. *Ecotoxicol and Environ Safety* 45, 198-207.

Buchet, J.P., Lauwerys, R. 1981. Evaluation of exposure to inorganic arsenic in man. In: Analytical Techniques for Heavy Metals in Biological Fluids, pp. 75-89. Elsevier. Amsterdam.

Cai, J., DuBow, M.S. 1997. Use of luminescent bacterial sensor for biomonitoring and characterization of arsenic toxicity of chromated copper arsenate (CCA). *Biodegradation* 8, 105-111.

Cai, Y., Cabrera, J.C., Geogiadis, M., Jayachandran, K. 2002. Assessment of arsenic mobility in the soils of some golf courses in South Florida. *Sci Tot Environ* (in press).

Capone, D.G., Reese, D., Kiene, R.P. 1983. Effects of metals on methanogenesis, sulfate reduction, carbon dioxide evolution and microbial biomass in anoxic salt marsh sediments. *Appl Environ Microbiol* 45, 1586-1591.

Carbonell-Barrachina, A.A., Jugsujinda, A., Burlo, F., Delaune, R.D., Patrick Jr., W.H. 1999. Arsenic chemistry in municipal sewage sludge as affected by redox potential and pH. *Wat Res* 34, 216-224.

Cervantes, C.; Ji, G.; Ramirez, J.L.; Silver, S. 1994. Resistance to arsenic compounds in microorganisms. *FEMS Microbiol Rev* 15, 355-367.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S., Baker, A.J.M. 1997. Phytoremediation of soil metals. *Current Opinion in Biotechnology* 8, 279-284.

Chang, Y-J., Peacock, A.D., Long, P.E., Stephen, J.R., McKinley, J.P., MacNaughton, S.J., Hussain, A.K.M.A., Saxton, A.M., White, D. 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. *Appl Environ Microbiol* 67, 3149-3160.

Chen, H.M., Zheng, C.R., Tu, C., Shen, Z.G. 2000. Chemical methods and phytoremediation of soil contaminated with heavy metals. *Chemosphere* 41, 229-234.

Cheng, C.N., Focht, D.D. 1979. Production of arsine and methylarsine in soil and culture. *Appl Environ Microbiol* 38, 494-498.

Chew, I., Obbard, J.P., Stanforth, R.R. 2001. Microbial cellulose decomposition in soils from a rifle range contaminated with heavy metals. *Environ Pollution* 111: 367-375.

Clement, B.G., Kehl, L.E., DeBord, K.L., Kitts, C.L. 1998. Terminal restriction fragment patterns (TREPs), a rapid PCR-based method for the comparison of complex bacterial communities. *J Microbiol Methods* 31, 135-142.

Compeau, G.C., Bartha, R. 1985. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediments. *Appl Environ Microbiol* 50, 498-502.

Cooper, E.M., Sims, J.T., Cunningham, S.D., Huang, J.W., Berti, W.R. 1999. Chelate-assisted phytoextraction of lead from contaminated soils. *J Environ Qual* 28, 1709-1719.

Corbisier, P., Ji, G., Nuyts, G., Mergeay, M., Silver, S. 1993. *luxAB* gene fusions with the arsenic and cadmium resistance operons of *Staphylococcus aureus* plasmid pI258. *FEMS MicrobiolLetters* 110, 231-238.

Corbisier, P., van der Lelie, D., Borremans, B., Provoost, A., de Lorenzo, V., Brown, N.L., Lloyd, J.R.,

Hobman, J.L., Csöregi, E., Gillis, J., Matiasson, B. 1999. Whole cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. *Analytica Chimica Acta* 387, 235-244.

Cotter-Howells, J.D., Caphorn, S. 1996. Remediation of contaminated land by formation of heavy metal phosphates. *Appl Geochem* 11, 335-342.

Cullen, W.R., Reimer, K.J. 1989. Arsenic speciation in the environment. *Chem. Rev.* 89: 713-764.

Dahlin, S., Witter, E., Mårtensson, A., Turner, A., Bååth, E. 1997. Where´s the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. *Soil Biol Biochem* 29, 1405-1415.

Dahmani-Muller, H., van Oort, F., Gelie, B., Balabane, M. 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environ Pollution* 109, 231-238.

Dahmani-Muller, H., van Oort, F., Balabane, M. 2001. Metal extraction by *Arabidopsis halleri* grown on an unpolluted soil amended with various metal-bearing solids: a pot experiment. *Environ Pollution* 114, 77-84.

Davis, A., Sherwin, D., Ditmars, R., Hoenke, K.A. 2001. An analysis of soil arsenic records of decision. *Environ Sci Technol* 12, 2401-2406.

Derome, J. 2000. Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilization. *Environ Pollution* 17, 79-88.

Deshpande, L.M., Kapadnis, B.P., Chopade, B.A. 1993. Metal resistance in *Acinetobacter* and its relation to beta-lactamase production. *Biometals: an International Journal on the Role of Metal Ions in Biology, Biochemistry and Medicine* 6, 55-59.

Diorio, C., J. Cai, J. Marmor, R. Shinder, and M. S. DuBow. 1995. An *Escherichia coli* chromosomal ars operon homolog is functional in arsenic detoxification and is conserved in gram-negative bacteria. *J Bacteriol* 177, 2050-6.

Doelman, P., Haanstra, L. 1979. Effects of lead on the soil bacterial microflora. *Soil Biol and Biochem* 11, 487-491.

Doelman, P., Haanstra, L. 1984. Long-term and short-term effects if cadmium, chromium and zinc on soil microbial respiration in relation to abiotic factors. *Plant and Soil* 79, 317-327.

Doelman, P. 1986. Resistance of soil microbial communities to heavy metals. In: Jensen, V., Kjoller, A., Sorensen, C.H (eds.) Microbial communities in soil. Elsevier Applied Science Publishers, London, UK.

Dungan. R.S., Frankenberger Jr., W.T. 2000. Factors affecting the volatilization of dimethylselenide by *Enterobacter clocae* SLD 1a-1. *Soil Biol Biochem* 32, 1353-1358.

Duxbury, T., Bicknell, B. 1983. Metal-tolerant bacterial populations from natural and metal-polluted soils. *Soil Biol Biochem* 15, 243-250.

Endo, G., Silver, S. 1995. CadC, the transcriptional regulatory protein of the cadmium resistance system of *Staphylococcus aureus* plasmid pI258. *J Bacteriol* 177, 4437-4441.

Ewers, U., Schlipköter, H-W. 1991. Lead. In: Merian, E. (Ed.) Metals and their compounds in the environment. VCH, Weinheim.

Faegri, A., Torsvik, V., Goksoyr, J. 1977. Bacterial and fungal activities in soil: separation of bacteria and fungi by a rapid fractionated centrifugation technique. *Soil Biol Biochem* 9, 105-112.

Fang, M., Wong, J.W.C. 1999. Effects of lime amendement on availability of heavy metals and maturation in sewage sludge composting. *Environ Pollution* 106, 83-89.

Fliessbach, A., Martens, R., Reber, H.H. 1994. Soil microbial biomass and microbial activity in soil treated with heavy metal contaminated sewage sludge. *Soil Biol Biochem* 26, 1201- 1205.

Flury, M., Frankenberger Jr., W.T., Jury, W.A. 1997. Long-term depletion of selenium from Kesterson dewatered sediments. *Sci Tot Environ* 198, 259-270.

Francis, A.J. 1998. Biotransformation of uranium and other actinides in radioactive wastes. *Journal of Alloys and Compounds* 271-273, 78-84.

Friedland, A.J., Bradley, W.C., Miller, E.K., Herrick, G.T., Siccama, T.G., Johnson, A.H. 1992. Decreasing lead levels in the forest floor of the north-eastern USA. *Ambio* 21, 400-403.

Frostegård, Å., Tunlid, A., Bååth, E. 1993. Phospholipid fatty acid compostion, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl Environ Microbiol* 11, 3605-3617.

Gadd, G.M. 1993. Microbial formation and transformation of organometallic and organometalloid compounds. *FEMS Microbiol Rev* 11, 297-316.

Gadd, G.M., 2000. Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology* 11, 271-279.

Gao, S., Burau, R.G. 1997. Environmental factors affecting rates of arsine evolution from and mineralization of arsenicals in soil. *J Environ Qual* 26, 753-763.

Garbisu, C., Alkorta, I. 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment (a review). *Bioresource Technology* 77, 229-236.

Gebel, T. 2000. Confounding variables in the environmental toxicology of arsenic. *Toxicology* 144, 155-162.

Gihring, T.M., Druschel, G.K., McCleskey, R.J., Hamers, R.J., Banfield, J.F. 2001. Rapid arsenite oxidation by *Thermus aquaticus* and *Thermus thermophilus*: Field and laboratory investigations. *Environ Sci Technol* 35, 3857-3862.

Gihring, T.M., Banfield, J.F. 2001 Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. *FEMS Microbiol Lett* 10169, 1-6.

Giller, K.E., Witter, E., McGrath, S.P. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a rewiev. *Soil Biol Biochem* 30, 1389-1414.

Groudev, S.N., Spasova, I.I., Georgiev, P.S. 2001. In situ bioremediation of soils contaminated with radioactive elements and toxic heavy metals. *Int J Miner Process* 62, 301-308.

Guo, L., Frankenberger, W.T., Jury, W.A.. 1999. Adsorption and degradation of dimethyl selenide in soil. *Environ Sci Technol* 33, 2934-2938.

Gupta, S.K., Vollmer, M.K., Krebs, R. 1996. The Importance of mobile, mobilisable and pseudo total heavy metal fractions in soil for three level risk assesement and risk management. *Sci Tot Environ* 178, 189-200.

Haavisto, T. 2002. Contaminated sites in Finland – Overview 2001. The Finnish environment series, Helsinki, Finland (in press).

Häggblom, M., Knight, V. and Kerkhof, L. 2000. Anaerobic decomposition of halogenated aromatic compounds. *Environ Pollution* (Special Issue). 107, 199-207

Hansen, L.H., Sorensen, S.J. 2000. Versatile biosensor vectors for detection and quantification of mercury. *FEMS Microbiol Letters* 193, 123-127.

Harter, R.D. 1979. Adsorption of copper and lead by Ap and B2 horizons of several Northeastern United States soils. *Soil Sci Soc Am J* 43, 679-683.

Hartley, J., Cairney, J.W.G., Freestone, P., Woods, C., Meharg, A.A. 1999. The effects of multiple metal contamination on ectomycorrhizal Scots pine (*Pinus sylvestris*) seedlings. *Environ Pollution* 106, 413-424.

Hattori, H. 1992. Influence of heavy metals on soil microbial activities. *Soil Science and Plant Nutrition* 38, 93-100.

Heinrichs, H., Mayer, R. 1980. The role of forest vegetation in the biogeochemical cycle of heavy metals. *J Environ Qual* 9, 111-118.

Hingston, J.A., Collins, C.D., Murphy, R.J., Lester, J.N. 2001. Leaching of chromated copper arsenate wood preservatives (a review). *Environ Pollution* 111, 53-66.

Hingston, J.A., Moore, J., Bacon, A., Lester, J.N., Murphy, R.J., Collins, C.D. 2002. The importance of the short-term leaching dynamics of wood preservatives. *Chemosphere* (in press).

Hiroki, M. 1992. Effects of heavy metal contaminationon soil microbial population. *Soil Science and Plant Nutrition* 38, 141-147.

Hongshao, Z., Stanforth, R. 2001. Competitive adsorption of phosphate and arsenate on goethite. *Environ Sci Technol* 35, 4753-4757.

Hozhina, E.I., Khramov, A.A., Gerasimov, P.A., Kumarkov, A.A. 2001. Uptake of heavy metals, arsenic and antimony by aquatic plants in the vincinity of ore mining and processing industries. *Journal of Geochemical Exploration* 74, 153-162.

Huang, J.W., Cunningham, S.D. 1996. Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol* 134, 75-84.

Huang, J.W., Chen, J., Berti, W.R., Cunningham, S.D. 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environ Sci Technol* 31, 800-805.

Huysmans, K.D., Frankenberger, W.T. 1991. Evolution of trimethylarsine by a *Penicillium* sp. isolated from agricultural evaporation pond water. *Sci Tot Environ* 105, 13-28.

Ilyaletdinov, A.N., Abdrashitova, S.A. 1981. Autotrophic oxidation of arsenic by a culture of *Pseudomonas arsenitoxidans. Mikrobiologiya* 50, 197-204.

Insam, H., Hutchinson, T.C., Reber, H.H. 1996. Effects of heavy metal stress on the metabolic quotient of the soil microflora. *Soil Biol Biochem* 28, 691-694.

Jain, C.K., Ali, I. 2000. Arsenic: Occurrence, toxicity and speciation techniques. *Wat Res* 34, 4304-4312.

Ji, G., Silver, S. 1992. Regulation and expression of the arsenic resistance operon from *Staphylococcus aureus* plasmid pI258. *J Bacteriol* 174, 3684-3694.

Ji, G., Garber, E., Armes, L., Chen, C-M., Fuchs, J., Silver, S. 1994. Arsenate reductase of *Staphylococcus aureus* plasmid pI258. *Biochemistry* 33, 7294-7299.

Ji, G., Silver, S. 1995. Bacterial resistance mechanisms for heavy metals of environmental concern. *J Ind Microbiol* 14, 61-75.

Johnson, C., Siccama, T., Driscoll, C., Likens, G., Moeller, R. 1995. Changes in lead biogeochemistry in response to decreasing atmospheric inputs. *Ecological Applications* 5, 813-822.

Johnson, F.M. 1998. The genetic effects of environmental lead. *Mutation Recearch* 410, 123-140.

Jones, D.E.H., Ledingham, K.W.D. 1982. Arsenic in Napoleon´s wallpaper. *Nature* 299, 626-627.

Jordan, M., Lechevalier, M.P. 1975. Effects of zink-smelter emissions on forest soil microflora. *Canadian J Microbiol* 21, 1855-1865.

Kairesalo, T., Tuominen, L., Hartikainen, H., Rankinen, K. 1995. The role of bacteria in the nutrient exchange between sediment and water in a flow-through system. *Microbial Ecology* 29, 129-144.

Kalbitz, K., Wennrich, R. 1998. Mobilization of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter. *Sci Tot Environ* 209, 27-39.

Kelly, J.J., Häggblom, M., Tate, R.L. 1999. Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. *Soil Biol Biochem* 31, 1455-1465.

Kerkhof, L., Santoro, M., Garland, J. 2000. Response of soybean rhizosphere communities to human hygiene water addition as determined by community-level physiological profiling (CLPP) and terminal restriction fragment length polymorphism (TRFLP) analysis. *FEMS Microbiol Lett* 184, 95-101.

Khan, M., Scullion, J. 2000. Effect of soil on microbial responses to metal contamination. *Environ Pollution* 110, 115-125.

Klassen, S.P., McLean, J.E., Grossl, P.R., Sims, R.C. 2000. Fate and behavior of lead in soils planted with metal resistant species (river birch and smallwing sedge). *J Environ Qual* 29, 1826-1834.

Konopka, A., Zakharova, T., Bischoff, M., Oliver, L., Nakatsu, C., Turco, R.F. 1999. Microbial biomass and activity in lead-contaminated soil. *Appl Environ Microbiol* 65, 2256-2259.

Kozdroj, J., van Elsas, J.D. 2001. Structural diversity of microorganisms in chemically perturbed soil assessed by molecular and cytochemical approaches. *J Microbiological Methods* 43, 197-212.

Krisnamurti, G.S.R., Huang, P.M., Van Rees, K.C.J., Kozak, L.M., Rostad, H.P.W. 1995. Speciation of particulate-bound cadmium of soils and its bioavailability. *Analyst.* 120, 659-665.

Kumar, N., Dushenkov, V., Motto, H., Raskin, I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ Sci Technol* 29, 1232-1238.

Kunito, T., Kazutoshi, S., Goto, S., Hayashi, H., Oyaizu, H., Matsumoto, S. 2001. Copper and zinc affecting microorganisms in long-term sludge-amended soils. *Bioresource Technology* 79: 135-146.

La Forece, M.J., Hansel, C.M., Fendorf, S. 2000. Arsenic speciation, seasonal transformations and co-distribution with iron in a mine waste-influenced palustrine emergent wetland. *Environ Sci Technol* 34, 3937-3943.

Lechevalier, M.P. 1977. Lipids in bacterial taxonomy – a taxonomist´s view. *Crit Rev Microbiol* 5, 109-210.

Leonard, A. 1991. Arsenic. In: Merian, E. (Ed.) Metals and their compounds in the environment, 751-772. VCH, Weinheim.

Lewin, P.K., Hancock, G.V., Voynovich, P. 1982. Napoleon Bonaparte – no evidence of chronic arsenic poisoning. *Nature* 299, 627-628.

Liu, W.T., Marsh, T.L., Cheng, H.., Forney, L.J. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* 63, 4516-4522.

Lloyd, J.R., Lovley, D.R. 2001. Microbial detoxification of metals and radionuclides. *Current Opinion in Biotechnology* 12, 248-253.

Lloyd, J.R., Mabbett, A.N., Williams, D.R., Macaskie, L.E. 2001. Metal reduction by sulphate-reducing bacteria: physiological diversity and metal specificity. *Hydrometallurgy* 59, 327-337.

Lock, K., Janssen, C.R. 2001. Ecotoxicology of zinc in spiked artificial soils versus contaminated field soils. *Env Sci Technol* 35, 4295-4300.

Löser, C., Seidel, H., Hoffmann, P., Zehnsdorf, A. 2001. Remediation of heavy metal-contaminated sediments by solid-bed bioleaching. *Environmental Geology* 40, 643-650.

Lovley, D.R., Philips, E.J.P., Gorby, Y.A., Landa, E.R. 1991. Microbial reduction of uranium. *Nature* 350, 413-416.

Lovley, D.R., Coates, J.D. 1997. Bioremediation of metal contamination. *Current Opinion in Biotechnology* 8, 285-289.

Lyytikäinen, M., Sormunen, A., Peräniemi, S., Kukkonen, J. 2001. Environmental fate and bioavailability of wood preservatives in freshwater sediments near an old sawmill site. *Chemosphere* 44, 341-350.

Ma, L.Q., Komart, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D. 2001. A fern that hyperaccumulates arsenic. *Nature* 409, 579.

Macnaughton, S., Stephen, J.R., Chang, Y.J., Peacock A., Flemming, C.A., Leung, K.T., White, D.C. 1999. Characterization of metal-resistant soil eubacteria by polymerase chain reaction-denaturing gradient gel electrophoresis with isolation of resistant strains. *Can J Microbiol* 45, 116-124.

Macur, R.E., Wheeler, J.T., McDermott, T.R., Inskeep, W.P. 2001. Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings. *Environ Sci Technol* 35, 3676-3682.

Madejon, P., Murillo, J.M., Maranon, T., Cabrera, F., Lopez, R. 2001. Bioaccumulation of As, Cd, Cu, Fe and Lead in wild grasses affected by the Aznalcollar mine spill (SW Spain). *Sci Tot Environ* (in press).

Maliszewska, W., Dec, S., Wierzbicka, H., Wozniakowska, A. 1985. The influence of various heavy metal compounds on the development and activity of soil micro-organisms. *Environ Pollution (Series A)* 37, 195-215.

Marin, A.R., Masscheleyn, P.H., Patrick Jr., W.H. 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant and Soil* 139, 175-183.

Marin, A.R., Masscheleyn, P.H., Patrick Jr., W.H. 1993. Soil redox-pH stability of arsenic species and its influence on arsenic uptake by rice. *Plant and Soil* 152, 245-253.

Marsh, T.L. 1999. Terminal restiction fragment length polymorphism (t-RFLP): an emerging method for characterizing diversity among homologous populations of amplification products. *Current Opinion in Microbiology* 2, 323-327.

Martens, D., Suarez, D. 1999. Transformations of volatile methylated selenium in soil. *Soil Biol Biochem* 31, 1355-1361.

Masscheleyn, P.H., Delaune, R.D., Patrick, W.H. 1991. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environ Sci Technol* 25, 1414-1419.

McLaren, R.G., Naidu, J., Smith, J., Tiller, K.G. 1998. Fractionation and distribution of arsenic in soils contaminated by cattle dip. *J Environ Qual* 27, 348-354.

McCreadie, H., Blowes, D.W., Ptacek, C.J., Jambor, J.L. 2000. Influence of reduction reactions and solid-phase composition on porewater concentration of arsenic. *Environ Sci Technol* 34, 3159-3166.

Meagher, R.B. 2000. Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology* 3, 153-162.

Mejare, M., Bulow, L. 2001. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *TRENDS in Biotechnology* 19, 67-73.

Morera, M.T., Echeverria, J.C., Mazkiaran, C., Garrido, J.J. 2001. Isotherms and sequential extraction procedures for evaluating sorption and distribution of heavy metals in soils. *Environ Pollution* 113, 135-144.

Moyer, C., Dobbs, F., Karl, D. 1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* 60, 871-879.

Muller, A.K., Rasmussen, L.D., Sorensen, S.J. 2001. Adaptation of the bacterial community to mercury contamination. *FEMS Microbiol Lett* 10124, 1-5.

Mulligan, C.N., Yong, R.N., Gibbs, B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology* 60, 193-207.

Murray, P., Ge, Y., Hendershot, W.H. 2000. Evaluating three trace metal contaminated sites: a field and laboratory investigation. *Environ Pollution* 107, 127-135.

Newman, D.K., Kennedy, E.K., Coates, J.D., Ahmann, D., Ellis, D.J., Lovley, D.R., Morel, F.M.M. 1997. Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. *Arch Microbiol* 168, 380-388.

Newman, D.K., Ahmann, D., Morel, F.M.M. 1998. A brief rewiev of microbial arsenate respiration. *Geomicrobiology J* 15, 255-268.

Nies, D.H. 1999. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol* 51, 730-750.

Nies, D.H., Silver, S. 1995. Ion efflux systems involved in bacterial metal resistances. *J Ind Microbiol* 14, 186-199.

Nieto, J.J., Fernandez-Castillo, R., Marquez, M.C. Ventosa, A., Quesada, E., Ruiz-Berraquero, F. 1989. Survey of metal tolerance in moderately halophilic eubacteria. *Appl Environ Microbiol* 55, 2385-2390.

Niggemyer, A., Spring, S., Stackebrandt, E., Rosenzweig, R.F. 2001. Isolation and characterization of a novel As(V)-reducing bacterium: implications for arsenic mobilization and the genus *Desulfitobacterium. Appl Environ Microbiol* 67, 5568-5580.

Nikunen, E., Leinonen, R., Kultamaa, A. 1990. Environmental properties of chemicals. Ministry of the Environment, Environmental Protection Department, Research Report 91, pp. 685-689.

Nkhalambayausi-Chirwa, E., Wang, Y-T. 2001. Simultaneous chromium(VI) reduction and phenol degradation in a fixed-film coculture bioreactor: reactor performance. *Wat Res* 35, 1921-1932.

Nriagu, J.O. 1978. The biogeochemistry of Pb in the environment. Elsevier, Amsterdam.

Oremland, R.S., Dowdle, P.R., Hoeft, S., Sharp, J.O., Schaefer, J.K., Miller, L..G., Blum, J.S., Smith, R.L., Bloom, N.S., Wallschlaeger, D. 2000. Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. *Geochimica et Cosmochimica Acta* 64, 3073-3084.

Osborn, A., Bruce, K., Strike, P., Ritchie, D. 1997. Distribution, diversity and evolution of the bacterial mercury resistance *(mer)* operon. *FEMS Microbiol Rev* 19, 239-262.

Otte, M.T., Dekkers, M.J., Rozema, J., Broekman, R.A. 1992. Uptake of arsenic by *Aster tripolium* in relation to rhizosphere oxidation. *Can J Botany* 69, 2670-2677.

QuiIntana, M., Curutchet, G., Donati, E. 2001. Factors affecting chromium(VI) reduction by *Thiobacillus ferrooxidans*. *Biochemical Engineering Journal* 9, 11-15.

Pan-Hou, H.S., Imura, N. 1982. Involvement of mercury methylation in microbiological mercury detoxification. *Arch Microbiol* 131, 176-177.

Pantsar-Kallio, M., Manninen, P.K.G. 1997. Speciation of mobile arsenic in soil samples as a function of pH. *Sci Tot Environ* 204, 193-200.

Pantsar-Kallio, M., Korpela, A. 2000. Analysis of gaseous arsenic species and stability studies of arsine and trimethylarsine by gas-chromatography-mass spectrometry. *Analytica Chimica Acta* 410, 65-70.

Peijnenburg, W.J.G.M., Posthuma, L., Eijsackers, H.J.P., Allen, H.E. 1997. A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicol and Environ Safety* 37, 163-172.

Pennanen, T., Frostegård, Å., Fritze, H., Bååth, E. 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal polluted gradients in coniferous forests. *Appl Environ Microbiol* 62, 420-428.

Pennanen, T., Perkiömäki, J., Kiikilä, O., Vanhala, P., Neuvonen, S., Fritze, H. 1998. Prolonged, simulated acid rain and heavy metal deposition: separated and combined effects on forest soil microbial community structure. *FEMS Microbiol Ecol* 27, 291-300.

Petänen, T., Virta, M., Karp, M., Romantschuk, M. 2001. Construction and use of broad host range mercury and arsenite sensor plasmids in the soil bacterium *Pseudomonas fluorescens* OS8. *Microb Ecol* 41, 360-368.

Phelps, C., L. Kerkhof, and L. Young. 1998. Molecular Characterization of a sulfate-reducing consortium which mineralizes benzene. *FEMS Microbiol Ecol* 27, 269-279

Plette, A.C.C., Nederlof, M.M., Temminghoff, E.J.M., van Riemsdijk, W.H. 1999. Bioavailability of heavy metals in terrestrial and aquatic systems: a quantitative approach. *Environ Toxicol Chem* 18, 1882-1890.

Polemio, M., Senesi, N., Bufo, S.A. 1982. Soil contamination by metals. A survey in industrial and rural areas of Southern Italy. *Sci Tot Environ* 25, 71-79.

Pongratz, R. 1998. Arsenic speciation in environmental samples of contaminated soil. *Sci Total Environ* 224, 133-141.

Pongratz, R., Heumann, K.G. 1999. Production of methylated mercury, lead and cadmium by marine bacteria as a significant natural source for atmospheric heavy metals in polar regions. *Chemosphere* 39, 89-102.

Ramanathan, S., Ensor, M., Daunert, S. 1997. Bacterial biosensors for monitoring toxic metals. *Trends in Biotechnology* 15, 500-506.

Raskin, I., Smith, R.D., Salt, D.E. 1997. Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology* 8, 221-226.

Rasmussen, L., Sorensen, S., Turner, R., Barkay, T. 2000. Application of *mer-lux* biosensor for estimating bioavailable mercury in soil. *Soil Biol Biochem* 32, 639-646.

Rasmussen, L.D., Sorensen, S.J. 2001. Effects of mercury contamination on the culturable heterotrophic, functional and genetic diversity of the bacterial community in soil. *FEMS Microbiol Ecol* 36, 1-9.

Reddy, K.J., Wang, L., Gloss, S.P. 1995. Solubility and mobility of copper, zinc and lead in acidic environments. *Plant and Soil* 171, 53-58.

Reimann, C., Koller, F., Frengstad, B., Kashulina, G., Niskavaara, H., Englmaier, P. 2001. Comparison of the element composition in several plant species and their substrates from a 1500000 km^2 area in Northern-Europe. *Sci Tot Environ* 278, 87-112.

Rensing, C., Ghosh, M., Rosen, B. 1999. Families of soft-metal-ion-transporting ATPases. *J Bacteriol* 181, 5891-5897.

Ridley, W.P., Dizikes, L.J., Wood, J.M. 1977. Biomethylation of toxic elements in the environment. *Science* 197, 329-332.

Roane, T.M., Kellogg, S.T. 1996. Characterization of bacterial communities in heavy metal contaminated soils. *Can J Microbiol* 42, 593-603.

Ross, S. 1994. Toxic metals in Soil-Plant Systems. John Wiley & Sons, Chichester, UK.

Ruokolainen, M., Pantsar-Kallio, M., Haapa, A., Kairesalo, T. 2000. Leaching, runoff and speciation of arsenic in a laboratory mesocosm. *Sci Total Environ* 258, 139-147.

Russeva, E. 1995. Speciation analysis – pecularities and requirements. *Anal Lab* 4, 143-148.

Rytuba, J. 2000. Mercury mine drainage and processes that control its environmental impact. *Sci Tot Environ* 260, 57-71.

Räisänen, M.L., Kashulina, G., Bogatyrev, I. 1997. Mobility and retention of heavy metals, arsenic and sulphur in podzols at eight locations in northern Finland and Norway and the western half of the Russian Kola Peninsula. *J Geochem Exploration* 59, 175-195.

Römkens, P., Bouwman, L., Japenga, J., Draaisma, C. 2002. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. *Environ Pollution* 116, 109-121.

Sadiq, M. 1997. Arsenic chemistry in soils: An overview of thermodynamic predictions and field

observations. *Water, Air and Soil Pollution* 93, 117-136.

Said, W.A., Lewis, D.L. 1991. Quantative assessment of the effects of metals on microbial degradation of organic chemicals. *Appl Environ Microbiol* 57, 1498-1503.

Sakano, Y., Kerkhof, L. 1998. Assessing changes in microbial community structure during operation of an ammonia biofilter using molecular tools. *Appl Environ Microbiol* 64, 4877-4882

Salt, D.E., Blaylock, M., Kumar, N., Dushenkov, V., Ensley, B.D., Chet, I., Raskin, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* 13, 468-474.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory Press, Cold Spring Harbour.

Sandaa, R.A., Torsvik, V., Enger, O. 2001. Influence of long-term heavy metal contamination on microbial communties in soil. *Soil Biol Biochem* 33, 287-295.

Santini, J.M., Sly, L.I., Schnagl, R.D., Macy, J.M. 2000. A new chemolitoautotrophic arsenite-oxidizing bacterium isolated from a gold-mine: phylogenetic, physiological, and preliminary biochemical studies. *Appl Environ Microbiol* 66, 92-97.

Sauve, S., McBride, M., Hendershot, W.H. 1997. Speciation in lead contamianted soils. *Environ Pollution* 98, 149-155.

Sauve, S., McBride, M., Hendershot, W.H. 1998. Lead phosphate solubility in water and soil suspensions. *Environ Sci Technol* 32, 388-393.

Sauve, S.F. 1999. Chemical Speciation, solubility and bioavailability of lead, copper and cadmium in contaminated soils. Cornell University, Ithaca, New York, USA.

Seidel, H., Ondruschka, J., Morgenstern, P., Wennrich, R., Hoffmann, P. 2000. Bioleaching of heavy-metal contaminated sediments by indigenous *Thiobacillus* spp.: metal solubilization and sulfur oxidation in the presence of surfactants. *Appl Microbiol Biotechnol* 54, 854-857.

Selifonova, O., Burlage, R., Barkay, T. 1993. Bioluminescent sensor for detection of bioavailable Hg(II) in the environment. *Appl Environ Microbiol* 59, 3083-3090.

Shann, J. 1995. The role of plants and plant/microbial systems in the reduction of exposure. *Environmental Health Perspectives Supplements* 103, 13-15.

Smit, E., Leeflang, P., Wernars, K. 1997. Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. *FEMS Microbiol Ecol* 23, 249-261.

Sohrin, Y., Matsui, M., Kawashima, M., Hojo, M., Hasegawa, H. 1997. Arsenic biogeochemistry affected by eutrophication in lake Biwa, Japan. *Environ Sci Technol* 31, 2712-2720.

Stolz, J., Oremland, R. 1999. Bacterial respiration of arsenic and selenium. *FEMS Microbiol Rev* 23, 615-627.

Tabatabai, M.A., 1977. Effects of trace elements on urease activity in soils. *Soil Biol Biochem* 9, 9-13.

Tauriainen, S., Karp, M., Virta, M. 1997. Recombinant luminescent bacteria for measuring bioavailable arsenite and antimonite. *Appl Environ Microbiol* 63, 4456-4461.

Tauriainen, S., Karp, M., Chang, W., Virta, M. 1998. Luminescent bacterial sensor for cadmium and lead. *Biosensors and Bioelectronics* 13, 931-938.

Tauriainen, S., Virta, M., Chang, W., Lampinen, J., Karp, M. 1999. Measurement of firefly luciferase reporter gene activity from cells and lysate using *Escherichia coli* arsenite and mercury sensors. *Anal Biochem* 272, 191-198.

Tauriainen, S., Virta, M., Karp, M. 2000. Detecting bioavailable toxic metals and metalloids from natural water samples using luminescent sensor bacteria. *Wat Res* 10, 2661-2666.

Tebo, B.M., Ghiorse, W.C., van Waasbergen, L.G., Siering, P.L., Caspi, R. 1997. Bacterially-mediated

mineral formation: insights into manganese(II) oxidation from molecular genetic and biochemical studies. *Rev Mineral* 35, 225-266.

Tebo, B.M., Obraztsova, A.Y. 1998. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microb Letters* 162, 193-198.

Tessier, A., Camleadell, P.G.C., Bisson, M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* 51, 844-851.

Tibazarwa, C., Corbisier, P., Mench, M., Bossus, A., Solda, P., Mergeay, M., Wyns, L., van der Lelie, D. 2001. A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants. *Environ Pollution* 113, 19-26.

Tom-Petersen, A., Hosbond, C., Nybroe, O. 2001. Identification of copper-induced genes in *Pseudomonas fluorescens* and use of a reporter strain to monitor bioavailable copper in soil. *FEMS Microbiol Ecol* 38, 59-67.

Torsvik, V., Daae, F., Sandaa, R-A., Ovreås, L. 1998. Novel techniques for analysing microbial diversity in natural and perturbed environments. *J Biotechnol* 64, 53-62.

Tsai, K.J., Yoon, K.-P., Lynn, A.R. 1992. ATP-dependent cadmium transport by the *cadA* cadmium resistance determinant in everted membrane vesicles of *Bacillus subtilis*. *J Bacteriol* 174, 116-121.

Tuominen, L., Kairesalo, T., Hartikainen, H. 1994. Comparison of methods for inhibiting bacterial activity in sediment. *Applied and Environ Microbiol* 60, 3454-3457.

Ulkomaanaho, L., Starr, M., Mannio, J., Ruoho-Airola, T. 2001. Heavy metal budgets for two headwater forested catchments in background areas of Finland. *Environ Pollution* 114, 63-75.

van del Lelie, D., Schwitzguebel, J-P., Glass, D.J., Vangronsveld, J., Baker, A. 2001. Assessing phytoremediation´s progress in the United States and Europe. *Environ Sci Technol* 21, 446A-452A.

van der Sloot, H.A., Coomans, R.N.J., Hjelmar, O. 1996. Similarities in the leaching behaviour of trace contaminants from waste, stabilized waste, construction materials and soils. *Sci Tot Environ* 178, 111-126.

Virta, M., Lampinen, J., Karp, M. 1995. A luminescence-based mercury biosensor. *Anal Chem* 67, 667-669.

Visoottiviseth, P., Francesconi, K., Sridokchan, W. 2002. The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environ Pollution* (in press).

Wagner-Döbler, I., Lunsdorf, H., Lubbehusen, T., von Canstein, H., Li, Y. 2000. Structure and species compositions of mercury-reducing biofilms. *Appl Environ Microbiol* 66, 4559-4563.

Wang, E.X., Benoit, G. 1996. Mechanisms controlling the mobility of lead in the spodsols of a northern hadrwood forest ecosystem. *Environ Sci Technol* 30, 2211-2219.

Watanabe, M.E. 1997. Phytoremediation on the brink of commercialization. *Environ Sci Technol* 31, 182-186.

Wenzel, W.W., Kirchbaumer, N., Prohaska, T., Stingeder, G., Lombi, E., Adriano, D.C. 2001. Arsenic fractionation in soils using an improved sequential extraction procedure. *Analytica Chimica Acta* 436, 309-323.

White, C., Sayer, J.A., Gadd, G.M. 1997. Microbial solubilization and immobilization of toxic metals: key biochemical processes for treatment of contamination. *FEMS Microbiol Rev* 20, 503-516.

White, D.C., Flemming, C.A., Leung, K.T., Macnaughton, S.J. 1998. In situ microbial ecology for quantitative appraisal, monitoring and risk assessment of pollution remediation in soils, the subsurface, the rhizosphere and in biofilms. *J Microbiol Methods* 32, 93-105.

Widmer, F., Fliessbach, A., Laczko, E., Schulze-Aurich, J., Zeyer, J. 2001. Assessing soil biological characteristics: a comparison of bulk soil community DNA-, PLFA- and Biolog™-analyses. *Soil Biol Biochem* 33, 1029-1036.

Windham, L., Weis, J.S., Weis, P. Lead uptake, distribution, and effects in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). *Marine Pollution Bulletin* 42, 811-816.

Woolson, E.A.1977a. Generation of alkylarsines from soil. *Weed Sci* 25, 412-416.

Woolson, E. A. 1977b. Fate of arsenicals in different environmental substrates. *Environmental Health Perspectives* 19, 73-81.

Woolson, E.A., Kearney, P.C. 1973. Persistence and reactions of ^{14}C-cacodylic acid in soils. *Environ Sci Technol* 7, 47-50.

Zelles, L., Bai, Q., Ma, R., Rackwitz, R., Winter, K., Beese, F. 1994. Microbial biomass, metabolic activity and nutritional status determined from fatty acid patterns and polyhydroxybutyrate in agriculturally-managed soils. *Soil Biol Biochem* 26, 439-446.

Zelles, L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* 35, 275-294.

Zhang, Y.O., Frankenberger, W.T. 1999. Effects of soil moisture, depth and organic amendments on selenium volatilization. *J Environ Qual* 28, 1321-1326.

Yoon, K.P., Silver, S. 1991. A second gene in the *Staphylococcus aureus cadA* cadmium resistance determinant of plasmid pI258. *J Bacteriol* 173, 7636-7642.