RAMAS

Risk Assessment and Risk Management
Procedure for Arsenic in the Tampere Region

Arsenic Ecotoxicity in Soils

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ABSTRACT

Schultz, E. & Joutti, A. 2007. Arsenic Ecotoxicity in Soils. Geological Survey of Finland, Miscellaneous Publications, 53 pages, 13 figures, and 10 tables.

Part A of this report is a literature survey on arsenic ecotoxicity in terrestrial environments and Part B describes the results of ecotoxicologial studies from the RAMAS project. Results of the ecotoxicological studies will be used in the ecological risk assessment of arsenic in the Pirkanmaa region.

Arsenic is a relatively common, toxic, carcinogenic metalloid that poses a significant environmental health hazard. The largest current anthropogenic use of arsenic in Finland is as a wood preservative in the form of chromated copper arsenate (CCA). Arsenic occurs in several forms, often in compounds with other chemical elements. Arsenic as a soil contaminant has different chemical fractions depending on the contamination and soil type. Some of these chemical fractions of arsenic are bioavailable and can be absorbed by organisms that are dependent on soil physicochemical conditions (e.g. soil texture, soil type, particle size, cation exchange capacity, pH, temperature, amount of organic matter, phosphate content,).

The determination of the total chemical contents is not sufficient to evaluate the ecological risk of arsenic, and bioassays are useful tools in monitoring the effects of soil contamination. Solid phase or direct contact bioassays are suitable and practical for testing arsenic effects in terrestrial environments. These tests describe the direct biological effects of arsenic between soil and test organisms. Bioassays with earthworms and germination tests with plants represent solid phase tests. Earthworms are the key organisms responsible for the mixing of soil constituents, maintaining the fertility and structure of soils and recycling nutrients. Terrestrial plants are the primary producers, supporting all other life forms. These characteristics make earthworms and terrestrial plants representative organisms for monitoring and assessment of soil quality.

In the experimental part of this study, the objective was to examine arsenic contaminated soil sites with both chemical and ecotoxicological methods. Nineteen soil samples were collected from an old wood preservation plant, a mine tailings area and from areas where the natural background concentrations of arsenic are high. The total arsenic concentrations extracted with aqua regia varied from 3 mg/kg in natural soils to more than 4000 mg/kg in contaminated soils. The fraction of arsenic leached by ammonium acetate-EDTA, which is supposed to reflect the bioavailability, was 7 % for CCA –soils, 25 % for mine tailing samples and less than 3mg/kg for natural soils. The bioavailable fraction of arsenic was extracted with solution.

For ecotoxicity testing, two plant solid phase tests or germination tests (ryegrass *Lolium multiflorum* and lettuce *Lactuca sativa)* were used. The germination tests showed that rye grass germination was not significantly affected by the samples but the effects on lettuce germination were more evident (inhibition up to 70 %). Two survival and reproduction tests of soil invertebrates (earthworms *Eisenia fetida* and pot worm *Enchytraeus albidus)* were also used for soil samples. Earthworms were more sensitive than enchytraeids. CCA soils were the most toxic, and even natural soils showed some effects. Samples from mine tailing area were difficult to assay, because earthworms did not thrive in the material without dilution.

Aquatic bioassays describe the leaching potential of water-soluble arsenic compounds. Two aquatic tests, duckweed (*Lemna minor*) growth inhibition and an enzymatic *in vitro* test RET (reverse electron transport) test were used. Duckweed plants are small free floating plants, which take all nutrients directly from the water. Electron transport is a chain of reactions essential for energy production in the mitochondria of living cells. CCA soil and mine tailing samples significantly inhibited duckweed growth, while. the natural soils had only a minor effect. Almost all samples, especially CCA soils, were inhibited in the RET test.

In summary, CCA soils were the most toxic samples in both the solid phase tests and aquatic tests.

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Keywords: arsenic, ecotoxicity, contaminated soil, earthworms, plant tests, bioavailability, wood preservatives, mine tailing, Pirkanmaa, Finland

TIIVISTELMÄ

Schultz, E. & Joutti, A. 2007. Arsenic Ecotoxicity of Soils. Geologian tutkimuskeskus, Erikoisjulkaisut, Ramas-projektisarja, 53 sivua, 13 kuvaa ja 10 taulukkoa.

Tämä raportti koostuu kirjallisuuskatsauksesta (osa A), joka käsittelee arseenin terrestristä ekotoksikologiaa sekä kokeellisesta osasta (osa B), jossa esitetään RAMAS –projektissa tehtyjen ekotoksikologisten kokeiden tulokset. Kokeellisen osan tuloksia käytetään myös arseenin ekologisen riskinarviointiin Pirkanmaan alueella.

Arseeni on melko yleinen, syöpää aiheuttava, ympäristömyrkyllinen puolimetalli eli metalloidi. Arseenia on käytetty moniin eri tarkoituksiin, mutta Suomessa eniten puunkyllästysaineissa eli kromia, kuparia ja arseenia (CCA) sisältävissä aineissa. Arseeni esiintyy kemiallisesti useassa muodossa, usein muiden alkuaineiden kanssa. Maaperäeliöt voivat kerätä biosaatavassa muodossa olevaa arseenia. Vuorovaikutukseen eliöiden, maaperän ja arseenin välillä vaikuttavat useat tekijät, kuten arseenin kemiallinen muoto ja alkuperä, arseenin muuntuminen. Vuorovaikutukseen vaikuttavat myös maaperän rakenne, maatyyppi, raekoko, kationinvaihtokapasiteetti, pH, lämpötila, orgaanisen aineksen määrä sekä fosfaatin ja muiden yhdisteiden pitoisuus maassa.

Kemiallisten kokonaispitoisuuksien perusteella tehtyä arseenin ekologista riskin arviointia voidaan täydentää ekotoksikologisilla testeillä. Erityisen sopivina arseenin myrkyllisyyden mittaamiseen pidetään ns. kiinteäfaasi- eli suorakontaktitestejä. Kiinteäfaasitestejä ovat maaperäeläinten (kuten lierojen) kuolevuutta ja lisääntymistä mittaavat testit sekä kasvitestit. Lieroilla on olennainen rooli maaperän rakenteen muokkaajana ja ravinteiden kiertokulussa. Kasveilla on tärkeä tehtävä kaiken muun elämän ylläpitäjänä, jolloin ne lierojen ohella sopivat hyvin biologiseksi indikaattoriksi.

Kokeellisessa osassa tutkittiin kemiallisin ja ekotoksikologisin menetelmin yhdeksäntoista maanäytettä, jotka olivat vanhalta puunkäsittelyalueelta, kaivosten rikastehiekka-alueelta sekä alueilta, joilla oli todettu luontaisesti korkeita arseenipitoisuuksia. Kuningasvesiuutolla saadut kemialliset maanäytteiden kokonaisarseenipitoisuudet vaihtelivat luonnon maanäytteiden matalista pitoisuuksista (3 mg/kg) pilaantuneen maan korkeisiin pitoisuuksiin (> 4000 mg/kg). Ammoniumasetaatti-EDTA-uutolla mitattiin ns. biosaatavaa arseenia, jota saatiin 7 % CCA-maiden sisältämästä arseenista, 25 % kaivosten rikastehiekan sisältämästä arseenista ja luonnon maista erittäin vähän, alle määritysrajan.

Ekotoksikologisissa kiinteäfaasikokeissa käytettiin kahta itävyystestiä (raiheinää *Lolium multiflorum* ja salaattia *Lactuca sativa*). Kiinteäfaasi testit eli terrestriset testit kuvaavat arseenin suoria biologisia vaikutuksia maaperän ja testieliön välillä. Itävyystestien tulosten perusteella raiheinän itäminen aleni hieman (inhibitio <14 %), mutta salaatin itäminen väheni selvästi (suurimmillaan 70 %:n inhibitio). Lisäksi tutkittiin maanäyteiden vaikutusta kahden maaperän selkärangattoman, lieron (*Eisenia fetida* ja änkyrimadon *(Enchytraeus albidus*, kuolevuuteen ja lisääntymiseen. Lierot olivat änkyrimatoja herkempiä. Änkyrimatojen kuolevuuteen ei näytteillä ollut vaikutuksia, mutta lisääntyminen väheni sekä luontaisten näytteiden, CCA-maiden että kaivosten rikastehiekan läsnä ollessa. Lierojen lisääntymistestissä erot luontaisten maiden ja CCA-maiden välillä olivat selvemmät, ja CCA-maat osoittautuivat haitallisiksi ja luonnonmaillakin havaittiin lieviä vaikutuksia. Kaivosten rikastehiekka ei soveltunut laimentamattomana lierojen elinalustaksi.

Vesieliöiden käyttöön perustuvilla ns. akvaattisilla testeillä voidaan arvioida veden välityksellä tapahtuvien arseenin haittavaikutusten määrää. Tässä työssä käytettiin kahta vesiympäristön biotestiä, kelluvan pikkulimaskan (*Lemna minor*) kasvun estymistestiä ja entsymaattista (reverse electron transport, RET) *in vitro* testiä. CCA-maa ja kaivosjäte estivät selvästi pikkulimaskan kasvua, kun taas luonnon maiden vaikutus oli vähäinen. Lähes kaikki näytteet estivät RET-testiä, ja CCA-maat olivat erityisen myrkyllisiä.

Yhteenvetona voidaan todeta, että tutkituista arseenipitoisista maanäytteistä CCA-maat olivat kaikkein myrkyllisimpiä. Myrkyllisyyttä havaittiin sekä tutkittaessa maaperäeläimillä ja kasveilla, että vesiympäristön testeillä.

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Keywords: arseeni, ekotoksisuus, pilaantunut maa, lierot, kasvitestit, biosaatavuus, puunkyllästysaineet, kaivosjäte, Pirkanmaa, Suomi.

PREFACE

RAMAS (LIFE04 ENV/FI/000300) is a three-year project, which is jointly funded by the LIFE ENVIRONMENT – program, by the beneficiary, the Geological Survey of Finland (GTK), and by the partners: the Helsinki University of Technology (TKK), the Pirkanmaa Regional Environment Center (PREC), the Finnish Environment Institute (SYKE), the Agrifood Research Finland (MTT), Esko Rossi Oy (ER) and Kemira Kemwater (Kemira).

The acronym RAMAS arises from the project title "Risk Assessment and Risk Management Procedure for Arsenic in the Tampere Region". The project targets the whole Province of Pirkanmaa (also called the Tampere Region), which comprises 33 municipalities, and has 455 000 inhabitants (currently 28 and 469 000, respectively) within its area. Tampere, Finland's third largest city, is the economical and cultural center of the region.

The project aims to identify the various sources of arsenic in the target area, to produce a health and environmental risk assessment for the region and to present recommendations for prevention and remediation and water and soil treatment methods. This project is the first in Finland to create an overall, large-scale risk management strategy for a region that has both natural and anthropogenic contaminant sources.

The project's work is divided into logically proceeding tasks, which have responsible Task Leaders who coordinate the work within their tasks:
1. Natural arsenic sources (GTK), Birgitta Backman
2. Anthropogenic arsenic sources (PREC), Kati Vaajasaari and Ämer Bilaletdin
3. Risk assessment (SYKE), Eija Schultz
4. Risk Management (SYKE), Jaana Sorvari
5. Dissemination of results (TKK), Kirsti Loukola-Ruskeeniemi
6. Project management (GTK), Timo Ruskeeniemi

The project produces a number of Technical Reports, which are published as a special series by the GTK. Each report will be an independent presentation of the topic of concern. More comprehensive conclusions will be drawn in the RAMAS project Final Report, which summarizes the project's results. Most of the reports will be published in English with a Finnish summary.

A cumulative list of the reports published so far will be given on the back cover of each report. All documents can be also downloaded from the project's home page: www.gtk.fi/projects/ramas.

1. INTRODUCTION

Arsenic is a metalloid and exhibits both metallic and nonmetallic properties. Arsenic is common as it ranks 20th in abundance in the earth's crust, 14th in seawater, and 12th in the human body (Bhumbla and Keefer 1994). Arsenic is found in many chemical forms and is transformed by environmental processes. It is also used in several industrial applications. Arsenic is common in the smelting industry, in which arsenic is a byproduct of ores containing Pb, Au, Zn, Co and Ni. Arsenic is also increasing in the microelectronics industry. Inorganic arsenic compounds have been widely used in products such as wood preservatives, pesticides and paints (Baird 1999; Mandal and Suzuki 2002). There has been growing concern about the environmental effects resulting from arsenic compounds and, therefore, many countries have set restrictions on the use of arsenic bearing materials.

Since arsenic is a normal constituent of the environment, there is a need for effective monitoring and measurement of arsenic at arsenic-containing soil and waste sites and at sites where arsenic occurs naturally in elevated concentrations. The analysis of soil samples should include identifying both the total amount of arsenic present and the specific chemical forms present. Arsenic speciation has acquired great importance in recent years, since the toxicity of arsenic differs strongly with the wide range of its organic and inorganic chemical forms (Cullen and Reimar 1989; Garcia-Manyes *et al.* 2002; Turpeinen *et al*. 2002).

Environmental assessment of contaminated soils is usually based on chemical analysis. However, chemical measurements alone are not sufficient to describe the risk of contaminated soils. Chemical data alone does not allow for an evaluation of the combined effects of the compounds present at a contaminated soil site. Bioassays can help to define the bioavailability and effects of environmental contaminants on biota. They integrate the biological effects, bioavailability, pH and interactions between arsenic and other compounds. Bioassays also help in evaluating the risks of contaminated soils. Various bioassays representing different trophic levels have been used for the screening of soil toxicity (Juvonen *et al*. 2000; Allen 2002; Vaajasaari *et al*. 2002; Schultz *et al.* 2004; Leitgib *et al*. 2006).

Test species used in laboratory experiments have to meet several requirements. A perfect test species would be a surrogate for many ecologically relevant species, easy to handle in laboratory conditions, well known for normal living habits and feed or nutrition, and exhibiting a rapid life cycle. For practical reasons, only a few species can be included into a test battery, which normally is composed of species from different trophic levels. Earthworms are important soil organisms and are the key organisms responsible for the mixing of soil constituents, maintenance of soil fertility and recycling of nutrients (Langdon *et al.* 2003).

Terrestrial plants are important for life because they are primary producers, and support all other life forms (Eapen and D'Souza 2005). The role of plants in soil development, stabilization, and nutrient cycling is essential. These characteristics make earthworms and terrestrial plants representative organisms for monitoring and assessment of soil quality. These tests represent direct contact tests or solid-phase tests and their main advantage is that interaction occurs between soil and test organisms, so that the mobility and bioavailability of the contaminant is included into the result.

In addition to these direct contact tests, aquatic bioassays are important to describe the leaching and mobility of toxic compounds from solid matter to the environment. Soil samples are extracted with water and the toxicity of the extracts are measured using aquatic organisms, like daphnids, algae,

and plants. Duckweed *Lemna* species have been used to test the toxicity of several elutriates from soil and waste.

This report contains two parts with the aim to:

- review the recent literature on the ecotoxicity of arsenic in terrestrial environments, especially research on toxic effects of arsenic compounds on earthworms and terrestrial plants (Part A - Literature review)

-examination of selected arsenic contaminated sites (Pirkanmaa region) using ecotoxicological methods and to determine the bioavailability of arsenic to earthworms in the study soils (Part B- Ecotoxicity testing with the RAMAS soil samples).

PART A - LITERATURE REVIEW

2. ARSENIC – A METALLOID IN SOILS

2.1. Chemical forms and biotransformation of arsenic

Arsenic is a relatively common, toxic, carcinogenic metalloid. It is widely distributed in nature and is commonly associated with metal ores. Arsenic is continually cycled through all environmental compartments (Fig. 1). It occurs in trace quantities in all rock, soil, water and air. The chemistry of arsenic is complex. Arsenic has four valency states –3, 0, +3 and +5. Arsenic occurs in several forms, often in compounds with other chemical elements (Table 1) (Allen 2002; Baroni *et al.* 2004; Garcia-Manyes *et al.* 2002; Masscheleyn *et al.* 1991). Under reducing conditions, arsenite (H_3AsO_3) is the dominant form and arsenate ($H_2AsO_4^-$ or $HAsO_4^{2-}$) is the stable form in oxygenated environments. Negatively charged arsenate is strongly adsorbed onto the surface of several common minerals. Arsenite adsorbs less strongly, a property that makes it more mobile (Mandal and Suzuki 2002).

A number of methylated organoarsenicals (methylarsonic acid MMA, dimethylarsenic acid DMA) broken down by biota, are found in nature (Table 1). These biomethylated arsenic compounds are formed in the soil–water, sediment-water interfaces through the activity of bacteria such as *Echerichia coli*, *Flavobacterium* sp, *Methanobacterium* sp, and fungi. Arsenic may also be converted to arsenobetaine and arsenic containing sugars, compounds that are found in high abundance in some marine animals and algae as well as terrestrial plants and animals (Baird 1999). The species-dependent toxicity of arsenic requires analytical techniques capable of distinguishing toxic from non-toxic chemical forms (Garcia-Manyes *et al.* 2002).

Table 1. Some commonly occurring arsenic compounds (according to Langdon *et al.* 2003).

Metallic arsenic	Arsenic compound
Arsenic trioxide (arsenous oxide)	As_2O_3 (As+3)
Arsenopyrite	FeAsS
Sodium arsenite	Na_3AsO_3 (As+3)
Arsenic trichloride	$AsCl_3$ (As+3)
Arsine gas	AsH_3 (As+3)
Arsenic acid	H_3AsO_4 (As+5)
Arsenates (lead and calcium)	$PbHAsO_4$ (As+5), $Ca_3(AsO4)_2$ (As+5)
Gallium arsenide	GaAs
Monosodium methane arsonate (MSMA)	(CH3)As+O(OH)(Ona)
Methylarsonic acid (MMA)	$CH_3AsO(OH)_2$
Dimethylarsenic acid (cacodylic acid, DMA)	$(CH3)_2AsO(OH)$
Arsenobetaine	$(CH3)_3As^+CH_2COO^-$
Arsenocholine (and other arsenolipids)	$(CH_3)_3As^+CH_2CH_2COH$

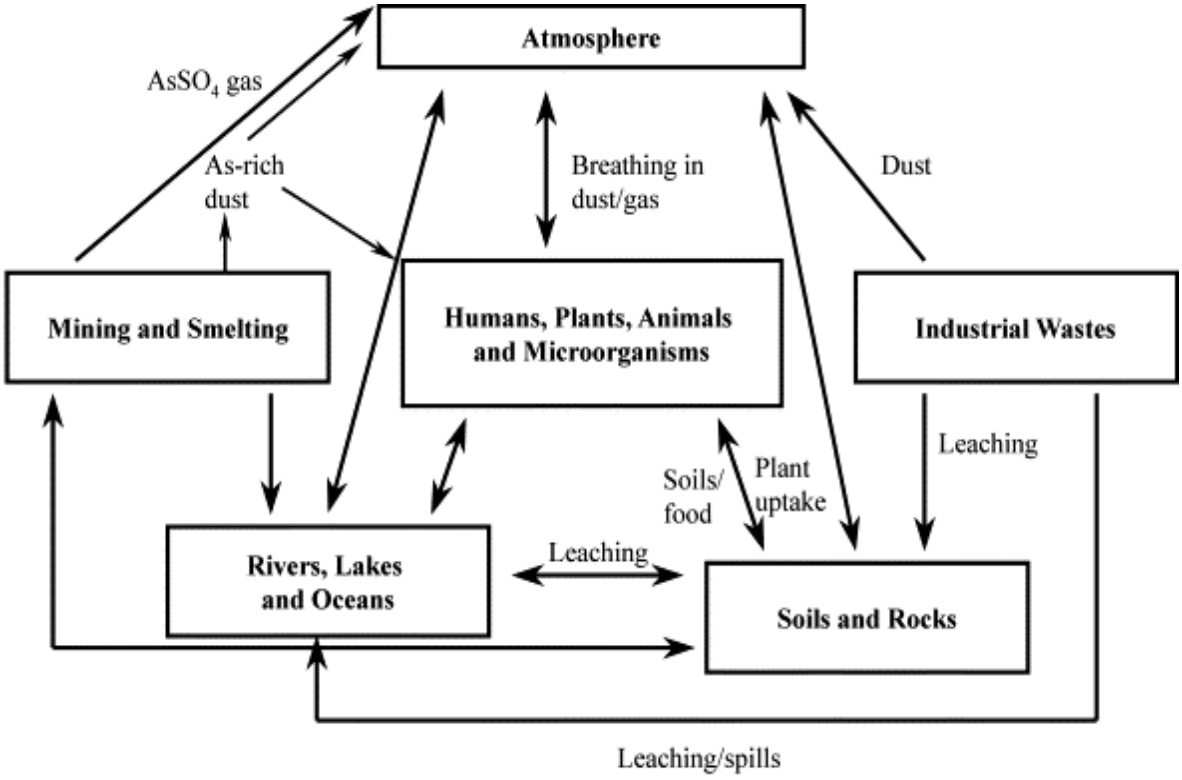

Figure 1. A simplified arsenic cycle (adapted from Bhumbla and Keefer 1994 and Langdon *et al.* 2003).

The biological availability and physiological and toxicological effects of arsenic depend on its chemical form. The species varies in toxicity and mobility. Arsenites are much more soluble, more mobile and more toxic than arsenates in soils. In general, organoarsenic compounds are less toxic than arsenates and arsenites forms (Allen 2002; Garcia-Manyes *et al.* 2002).

Most transformations of arsenic (biotransformation**,** biogeochemical cycling) occur in the soil, in sediments, in plants and animals, and in zones of biological activity in the oceans. Arsenic species are transformed by biological activity, changes in pH or changes in redox potential (Matera *et al.* 2003). Since the redox potential of soils depends on the redox potentials of all the reducing and oxidizing factors in the soils, all these systems are very complex. Three modes of arsenic biotransformation in the environment are:

- redox transformation between arsenite and arsenate;
- reduction and methylation of inorganic arsenic; and
- biosynthesis of more complex organic arsenic compounds.

Terrestrial plants (Fig. 1) accumulate inorganic arsenic by root uptake from the soil or by adsorption of airborne arsenic deposited on the leaves. Biomethylation and bioreduction are probably the most important environmental transformations of arsenic, since they can produce organometallic species that are sufficiently stable to be mobile in air and water. However, the biomethylated forms of arsenic are subject to oxidation and bacterial demethylation back to inorganic forms (Allen 2002; Baroni *et al.* 2004; Garcia-Manyes *et al.* 2002; Masscheleyn *et al.* 1991).

Table 2. Examples of industrial uses of arsenic (according to Baird 1999).

Industry	Compound	Use
Wood processing	Copper, chrome arsenate (CCA)	Preservation
Chemical	Arsenic trioxide, arsenic pentoxide, sodium arsenate	Manufacture of specialty chemicals
Pharmaceuticals	Arsenic trioxide	Manufacture of arsenilic acid, cacodylates
Glass	Arsenic trioxide	Decolorizing agent
Agriculture	Arsenic acid, cacodylic acid	Herbicides, pesticides, wood preservatives
Metallurgy	Arsenic trioxide	Hardens lead used in battery grids, bearing and cable sheathing
Semiconductor	Arsine gas, arsenic trioxide, gallium arsenide	Doping of chips

2.2. Industrial sources of arsenic

Arsenic has been used for several thousand years. Arsenic has a long history of medical applications. Before penicillin was developed, an organoarsenic compound, salvarsan (arsphenamine), was used to treat syphilis. A wide range of arsenicals was also used for the treatment of infectious diseases. Arsenic compounds have many industrial applications (Table 2).

Industrial arsenic sources include smelter slag, run off from mine tailings, coal or peat combustion, pigment production for paints and dyes, the processing of pressure-treated wood (CCA). From the 1930's to the 1980's, the application of arsenic-based herbicides and pesticides was very common. Arsenic-containing compounds are also used in electronics manufacturing. It has been estimated that 70% of the world arsenic production is used in wood treatment as copper chrome arsenate (CCA), 22% in agricultural chemicals, and the remainder in glass, pharmaceuticals and non-ferrous alloys (Mandal and Suzuki 2002).

At the moment, arsenic has been replaced in most applications by synthetic dyes and pesticides, but it is still used, for example, in agriculture. Organic arsenicals like roxarsone (4-hydroxy-3-nitrophenyl arsonic acid) used in U.S. act as an intestinal palliative for swine, improve pigmentation, and increase the growth of poultry. Arsenic does not accumulate in flesh, meat, or eggs but is excreted (Czarnecki and Baker 1982). The production and storage of chemical weapons, such as phenyldichloroarsine, diphenylchloroarsine, and diphenylcyanoarsine has also resulted in heavy contamination in some areas in Eastern Europe (Kohler *et al.* 2001).

If arsenic bearing material is burned or treated thermally, arsenic is volatilized (Fig. 1). A specific case is the thermal conversion of CCA-treated wood. CCA-treated wood contains a thousand times more arsenic than coal. Arsenic fumes are difficult to control in conventional air pollution control devices (Helsen and van den Bulck 2005).

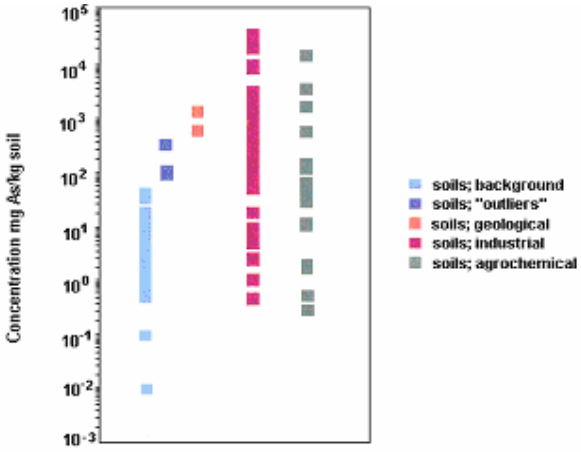

Figure 2. Reported "global" concentrations of arsenic in soils (http://www.inchem.org/documents/ehc/ehc/ ehc224.htm#1.0). Values plotted as “outliers” are the upper end of ranges where bedrock or freshwater sediments were thought to contribute to higher than normal arsenic levels. “Geological” values are for volcanic areas. “Industrial” values are for mining, smelting and manufacture of agrochemicals. The “agrochemicals” covers values following the use of pesticides, sheep dips, etc.

2.3. Concentrations in soils

Soils are not homogeneous and contain variable amounts of arsenic (Fig. 2). On a global scale, background concentrations in the soil range from 0.1- 40 mg/kg but vary among geographic regions. Soils exposed to industrial effluents and wastes, areas next to smelters and mine spoils have the greatest accumulation of arsenic (up to100 g/kg). The highest values of arsenic in soil are associated with mining waste. Use of arsenic-containing pesticides has left large tracts of agricultural land contaminated. The use of arsenic in the preservation of wood has also led to contamination of the environment (http://www.inchem.org/documents/ehc/ehc/ ehc224.htm#1.0). According to the new Finnish regulations the threshold limit value for arsenic in soils is 5 mg/kg. Cleanup levels for contaminated soil sites vary from 50 to 100 mg/kg of arsenic and cleanup level for hazardous waste sites is 1000 mg/kg (http://www.miljo.fi/download.asp?contentid=66591&lan=fi).

A European geochemical survey (FOREGS Geochemical Baseline Mapping Programme in Europe) reported mean arsenic concentrations of 9.88 mg/kg in topsoil (Salminen et al. 2005). Samples (n = 840) were taken from a depth of 0 - 25 cm and analyzed from grain size < 2 mm from aqua regia leach. In subsoil (depth 50 – 200 cm), the mean value was 9.75 mg/kg (n = 784).

In comparison to these reported data, RAMAS (Backman *et al.* 2006) described the occurrence and concentrations of natural arsenic in Pirkanmaa, Finland. Natural arsenic is derived from the arsenic bearing minerals, which are enriched in the bedrock. In Finland, the glaciogenic events were important in dispersing arsenic into the surrounding areas. The arsenic problem is focused in the Tampere Schist Belt (TB) and the Pirkanmaa Belt (PB), where metamorphosed volcanites comprise a major part of the bedrock. The arsenic concentrations in bedrock varied from 0.1 to 377 mg/kg.

According to Backman *et al.* 2006, till is the main soil type in Pirkanmaa and the median value for arsenic in Pirkanmaa was double compared to the rest of the country (5.3 mg/kg vs. 2.6 mg/kg). The highest median values in tills are encountered in the TB (5.92 mg/kg) and the PB (11.5 mg/kg). Arsenic concentrations tend to increase downwards in the soil profile (the highest concentrations are 9 280 mg/kg).

Parviainen *et al.* 2006 also reported that wood preservation plants are the major anthropogenic source for arsenic in the Pirkanmaa region, in Finland. Concentrations of arsenic in the soils of wood preservative plants range from 3 up to 12000 mg/kg. Shotgun shooting ranges are also possible arsenic contaminated areas (concentrations vary from 1.1 to 28 mg As /kg). The mining industry affects large areas through air and especially through surface waters. The samples from the abandoned Ylöjärvi mine tailing samples showed arsenic concentrations ranging from 1000 to 2200 mg /kg. Household waste material did not contain high concentrations of arsenic in Pirkanmaa. Moreover, old, poorly isolated landfills containing disposed CCA-treated wood or wood preservative product wastes, closed factories, old refineries, tanneries, and animal shelters are potentially arsenic contaminated areas. However, according to Parviainen *et al*. (2006), the concentrations of arsenic of the contaminated sites in Pirkanmaa are small in comparison to other European countries.

2.4. Assays for arsenic in the soil

Arsenic is common, as it is found in many chemical forms and is transformed by environmental processes (Fig. 3). It is also found as a result of several industrial applications. There is a need for effective monitoring and measurement of arsenic in soils and wastes for example. The analysis of soil samples should include identifying both the total amount of arsenic present and the specific chemical forms present (speciation).

2.4.1. Total content of arsenic

Fixed laboratory assays are generally required to accurately measure arsenic in an environmental sample to concentrations µg/kg for solids. The preferred laboratory methods for the measurement of arsenic involve pretreatment, either with acidic extraction or acidic oxidation digestion of the environmental sample. Pre-treatment transfers all of the arsenic in the sample into an arsenic acid solution, which is subsequently measured using any one of several accepted analytical methods, such as atomic graphite furnace atomic absorption (GFAA), hydride generation atomic absorption spectroscopy (HGAAS), inductively coupled plasma-atomic emission spectrometry (ICP-OES), and inductively coupled plasma-mass spectrometry (ICP-MS) (Melamed 2005).

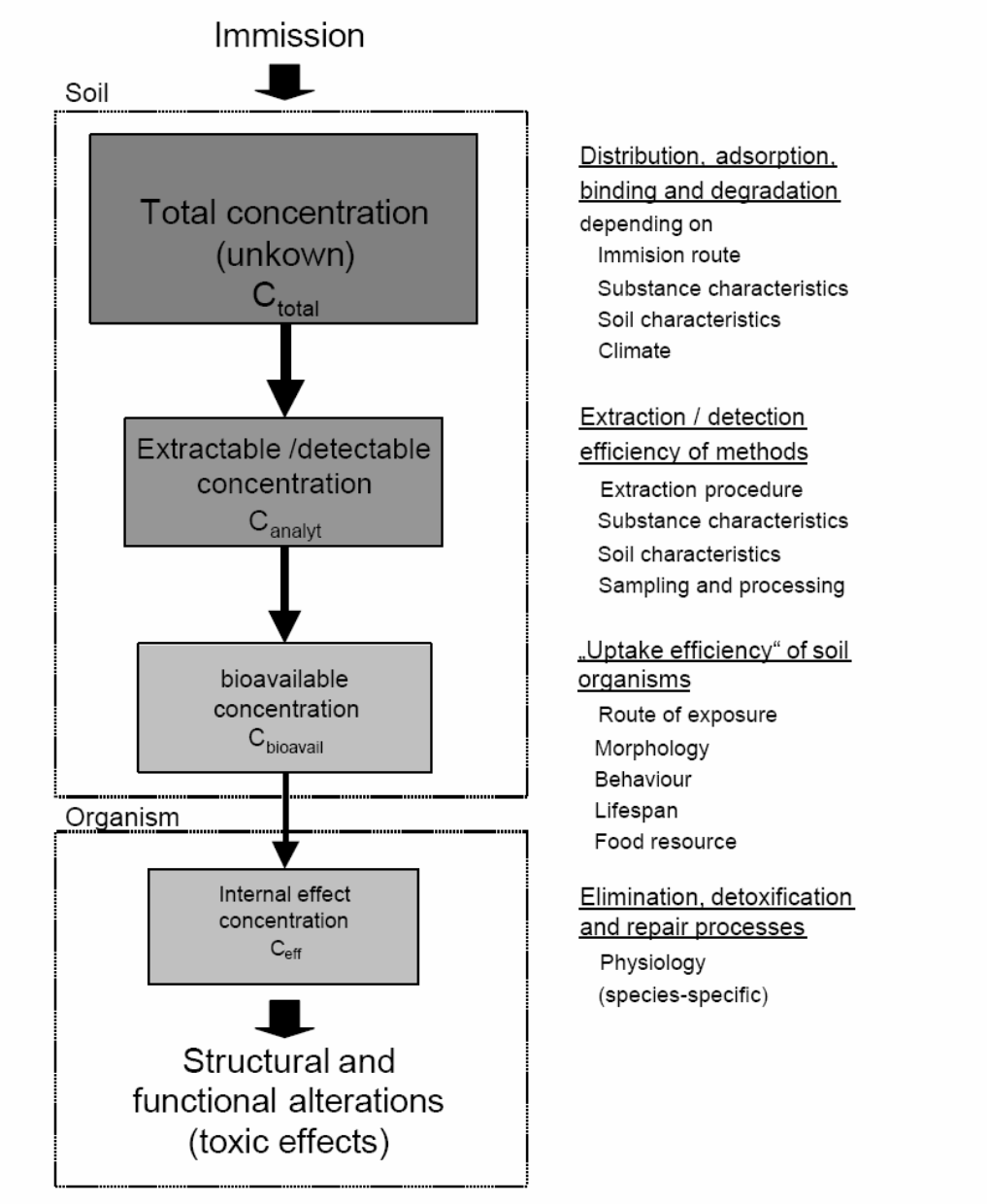

Figure 3. Contaminant flow-path in soil and organisms
http://www.umweltdaten.de/publikationen/fpdf-l/2373.pdf)

2.4.2 Arsenic species in soils

Mobility, bioavailability, and toxicity of arsenic depend strongly on their chemical form and type of binding (Fig. 3 and 4). Consequently, determination of total arsenic concentrations is insufficient to estimate toxicity and potential risks of arsenic.

Arsenate, arsenate anions, along with the neutral arsenite are the main targets for analytical assays. In contaminated soils, inorganic arsenate is the predominant species. In general, the arsenate and other arsenic (+5) species are immobilized on geologically available surfaces, often on iron (oxy)hydroxides. Although arsenic (+5) compounds are considered a low risk, bacterial activity can readily convert them back into more mobile and more toxic forms of arsenic. Soils also contain organoarsenic species: monomethylarsenic acid, dimethylarsenic acid, trimethylarsine oxide, and

trimethyl arsine. In general, organoarsenic compounds are less toxic than their corresponding oxyacids. Organoarsenic compounds are usually found in lower concentrations, however, under certain conditions, they can be found in very high concentrations in soils (Turpeinen *et al.* 1999; Cappuyns *et al.* 2002; Garcia-Manyes *et al.* 2002; Matera *et al.* 2003; Cepria *et al.* 2005). The species-dependent toxicity of arsenic requires analytical techniques capable of distinguishing toxic from non-toxic chemical forms.

The extraction of chemical species is a crucial topic in element speciation studies in complex matrices in which the extraction system has to provide good recovery and to preserve the identity of the native species in the soil sample. Accurate characterization of solid phases at contaminated sites plays a crucial role in risk assessment and risk management of inorganic pollutants (Matera *et al.* 2003; Cepria *et al.* 2005). However, analysis of species is expensive and care must be taken to ensure the preservation of the different arsenic speciation in a sample (Melamed 2005).

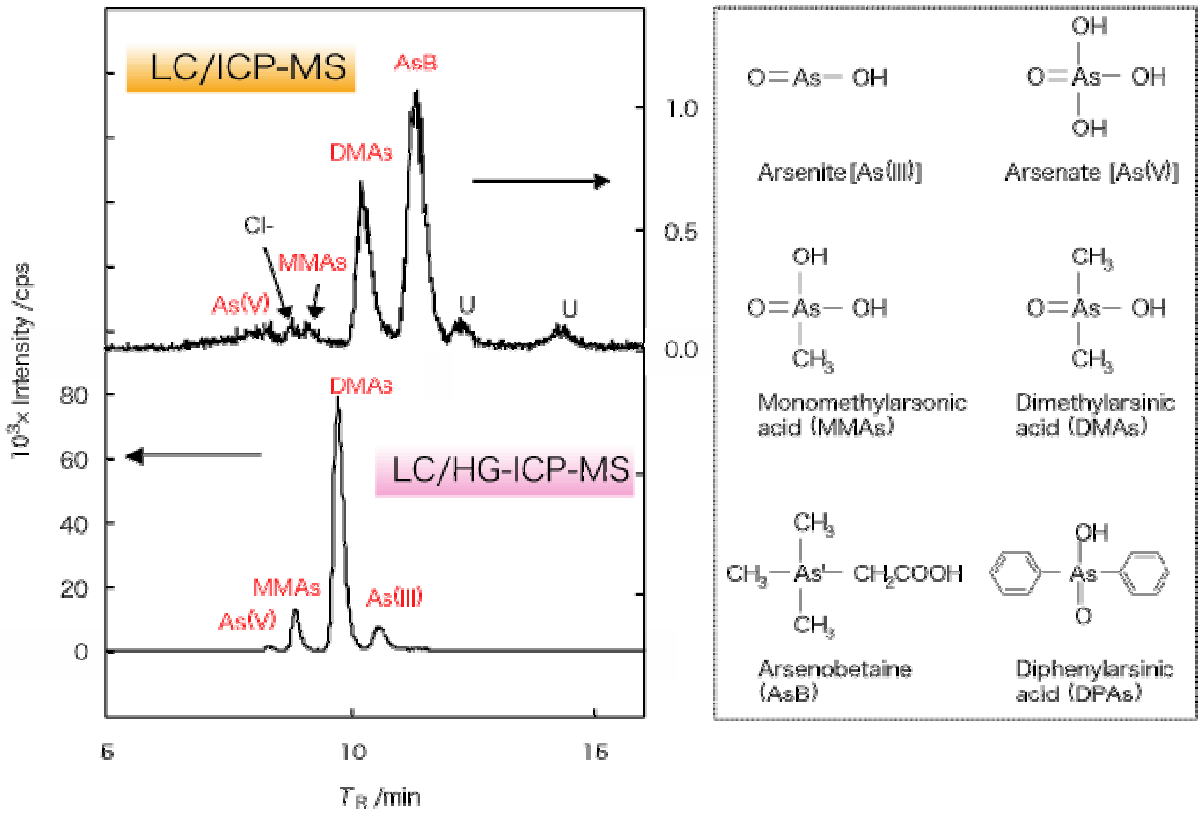

Figure 4. Arsenic species of an environmental sample and chromatographic separation (http://www.aist.go.jp/aist_e/aist_today/2004_13/feature2/feature_04.html). Please note that two abbreviations (MMAs, DMAs) are different than in Table 1 (MMA, DMA).

2.4.3. Field measurements

Arsenic is a permanent part of the environment, and there is a need for regular monitoring at soil/waste sites and at sites where it occurs naturally at elevated concentrations. A range of analytical field assays for pollutants such as arsenic provides valuable tools to support improved site characterization. Field assays, in which lower sensitivities may be acceptable for screening soil site surveys, strive for similar detection goals, are relatively inexpensive, and can produce a large number of screening results in a short period of time. Field methods for the analysis of arsenic in the environment are colorimetric test kits, portable X-ray fluorescence instruments, etc. (Melamed 2005).

2.4.4. Bioavailability

An important key to understanding the environmental risk from arsenic is also bioavailability, which is defined as the measure of the amount of arsenic that can be absorbed by a living organism. However, the bioavailability of metals into living organisms in soils is complex, and is not only affected by the total soil metal concentration, but also by the soil's physical and chemical characteristics, the kinetics of bioaccumulation, storage and excretion, and the organism's tolerance of the element concerned (Fig. 3). According to Frische *et al.* (2002):
"Bioavailability describes the complex processes of mass transfer and uptake of contaminants into soil-living organisms which are determined by substance properties, soil properties, the biology of organisms and climatic influences. The bioavailable contaminant fraction in soil represents the relevant exposure concentration for soil organisms."

Although bioavailability is likely to play a strong role in future environmental regulatory decisions, it has not received widespread regulatory and public acceptance. At present, there is no general concept that could unify the various theoretical and experimental methods for assessing bioavailability. Thus, the techniques for measuring bioavailable arsenic are varied and are the subject of ongoing research (Langdon *et al.* 2003; Melamed 2005). Bioavailability requires extensive further work, both in theory (concepts, models) and in practice (standardisation) (Frische *et al.* (2002).

3. BIOLOGICAL INTERACTIONS BETWEEN ARSENIC AND TERRESTRIAL BIOTA

Soil is a dynamic and complex system functioning as habitat for micro-organisms, flora, animals and humans (Fig. 5). Contaminated soils have become a problem since they will lead to, for example, groundwater contamination of chemical compounds through food webs, and sometimes will affect human health (Hund-Rinke *et al.* 2002).

Arsenic contamination of terrestrial ecosystems is widespread, arising from many various sources, including geological and anthropological sources. Each contaminated site has its own hazardous compounds and exposure routes to soil organisms through the food chain (Fig. 5). Plants, fauna and micro-organisms have different exposure routes (Table 3). The chemistry of As in biological systems is not well-known (Langdon *et al.* 2003).

Terrestrial plants may accumulate arsenic by root uptake from the soil or by adsorption of airborne arsenic deposited on the leaves. Arsenic levels are higher in biota collected near anthropogenic sources or in areas with geothermal activity or other natural sources. Some species accumulate

substantial levels, with mean concentrations of up to 3000 mg/kg at arsenical mine sites. Background arsenic concentrations of terrestrial biota are usually less than 1 mg/kg (fresh weight).

Reported total arsenic concentrations in soil can be very high. However, total arsenic is a poor indicator of toxicity to biota. Bioavailable arsenic represents a small fraction of total soil arsenic (10% or less and usually < 2%). On severely contaminated mine wastes, specialized arsenic tolerant plant communities have developed. Some tolerant plants grow on wastes with total arsenic levels of several percent by weight. However, communities are likely to be low in biodiversity at high arsenic concentrations.

Living organisms are exposed to many different forms of inorganic and organic arsenic species (arsenicals) in soils (Fig. 6). Each of the forms of arsenic has different physicochemical properties and bioavailability. The bioavailability of ingested inorganic arsenic varies depending on the matrix in which it is ingested (in water, in soil), the solubility of the arsenical itself and the presence of

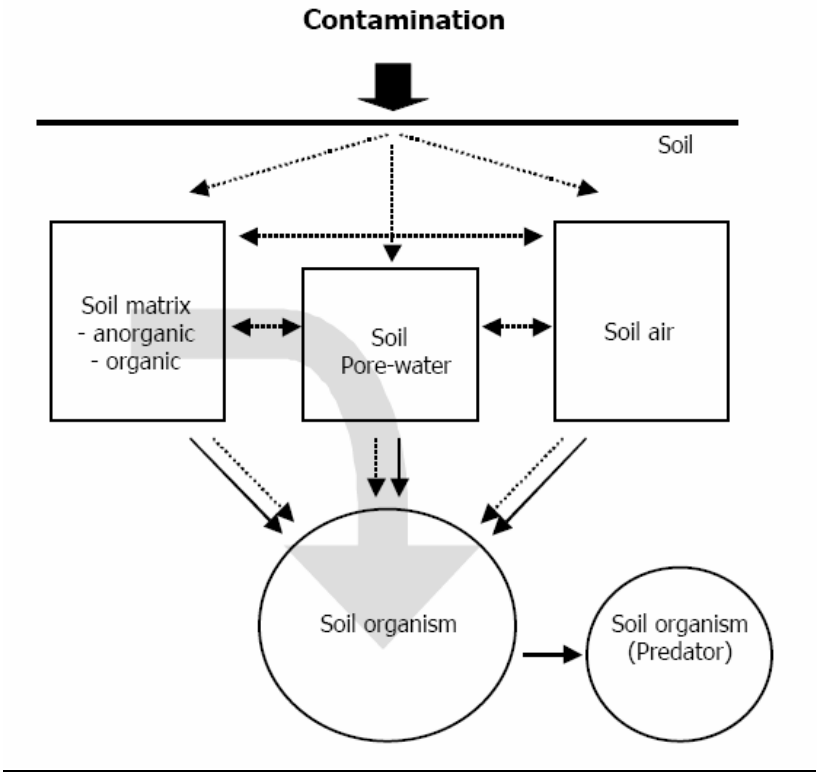

Figure 5. Model for soil and exposure routes for soil-living organisms (http://www.umweltdaten.de/publikationen/fpdf-l/2373.pdf).

Table 3. Exposure routes for organisms in terrestrial test systems (**http://www.umweltdaten.de/publikationen/fpdf-l/2373.pdf**).

Taxonomic group		**Species**	Soil solution	Soil air	Air near surface	Organic matter, dead	Inorganic matter	Organic matter, alive
Plants	Monocotyl	*Avena sativa*	++*)	(+)	(+)	-	-	-
	Dicotyl	*Brassica rapa*	++	(+)	(+)	-	-	-
Micro-organisms	Bacteria	mixed populations	++	-	-	(+)	(+)	-
	Fungi	mixed populations	++	-	-	(+)	(+)	-
	Protozoa	mixed populations	++	-	-	(+)	(+)	-
Fauna	Nematoda		++	-	-	-	-	+
	Enchytraeidae	*Enchytraeus albidus*	++	-	-	+	-	+
	Lumbricidae	*Eisenia fetida*	++	(+)	-	+		-
	Isopoda	*Porcellio scaber*	-	+	-	++	-	-
	Collembola	*Folsomia candida*	+	+	+	++	-	-
	Carabidae		-	+	+	-	-	++

*) ++ main exposure, + exposure, (+) subordinate significance, - no significance

compounds, membrane characteristics, etc. The fate of arsenical in vivo depends on oxidation and reduction between As(III) and As(V), etc. In most animal tests, DMA is the main metabolite. The metabolism of arsenicals in living organisms is very complex and not well-known.

Arsenic is toxic to terrestrial biota because it inhibits basic cellular functions linked with energy metabolism (Ghosh *et al.* 2004). The toxicity of arsenic is a consequence of its similarity to P in the As(V) form and its ability to form covalent bonds with S in the As(III) form. Arsenate, an analogue of the essential phosphate anion, is taken up by most organisms via their phosphate transport system. It has been hypothesized that arsenate replaces phosphate in energy-transfer phosphorylation reactions. Arsenite has a high affinity for the thiol groups of proteins and can thus inactivate many enzymes. As a result of these two mechanisms, arsenic can directly influence the biota present in soils and decreases in microbial populations have been reported in soils polluted with arsenic compounds (Maliszewska-Kordybach & Smreczak 2003; Ghosh *et al.* 2004).

The toxicity of arsenic varies according to environmental conditions, the arsenic compound and species. Inorganic arsenic and arsenic combined with oxygen, chlorine or sulfur are most toxic while most organic arsenic compounds are less toxic. Some of these chemical fractions are bioavailable and thus can be absorbed by organisms that are dependent of the soil physicochemical conditions (e.g. pH, clay content, cation exchange capacity, amount of organic matter) and on the chemical form of the element (Fig. 6). Therefore, the determination of the total chemical contents is not sufficient to evaluate the ecological risk that is inherent to a contaminated soil.

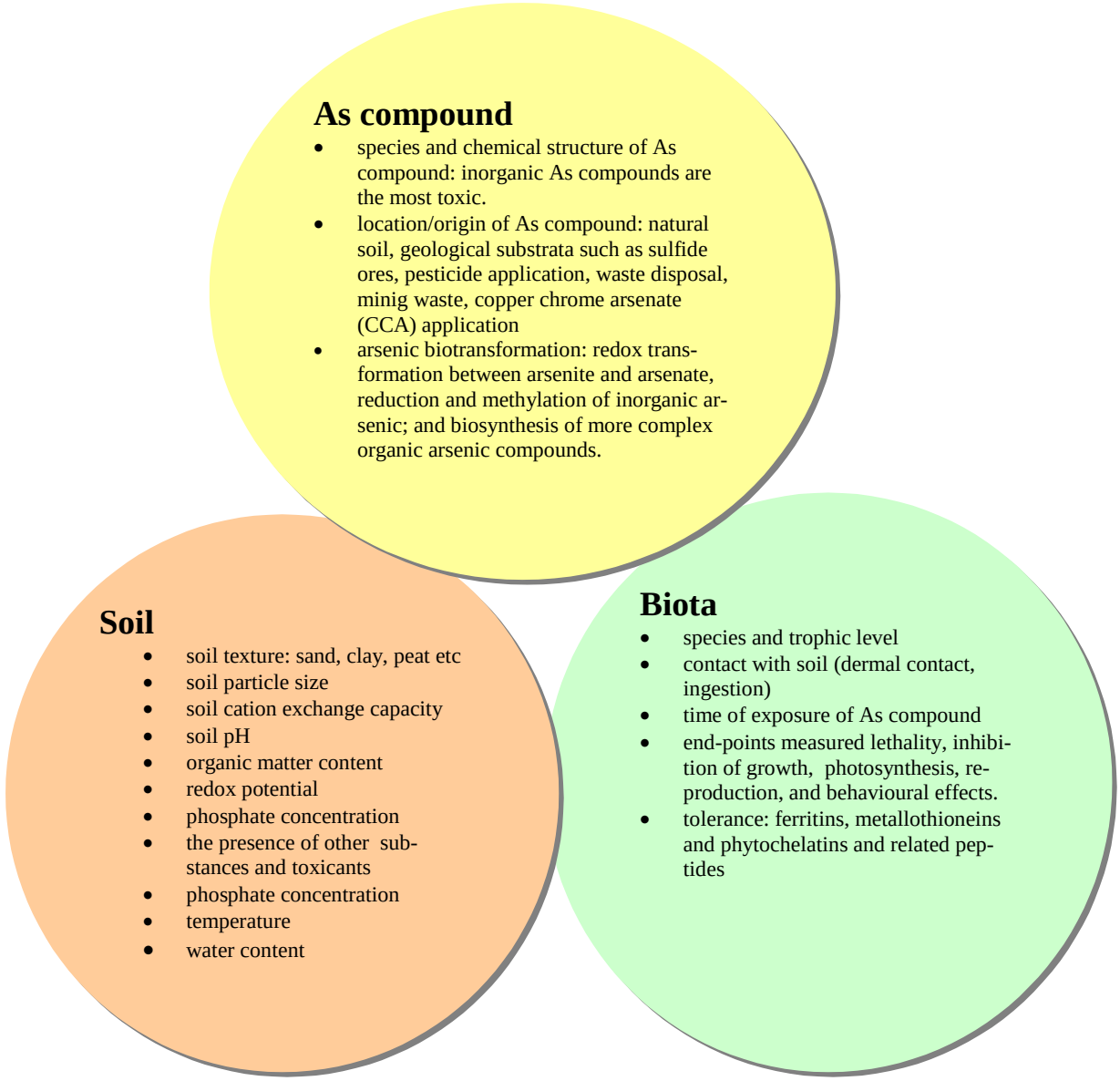

Figure 6. Parameters controlling the interactions between As, soil and biota.

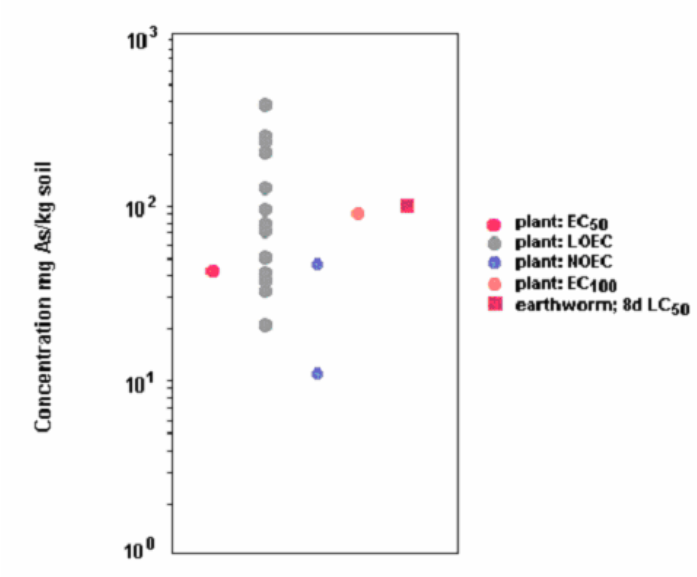

Figure 7. Terrestrial toxicity of arsenic and organic arsenic compounds used as pesticides. Values for plants are sublethal effects (growth or yield) http://www.inchem.org/documents/ehc/ehc/ehc224.htm#1.0.

Terrestrial toxicity of arsenic compounds used as pesticides is summarized in Fig. 7. Levels of soil arsenic reported to be toxic to plants ranged from 30 mg/kg to 300 mg/kg with toxicity tending to be greater in sandy than in clay soils. The lowest critical plant tissue concentration was around 1 mg/kg. Very limited data are available for soil invertebrates (http://www.umweltdaten.de/publikationen/fpdf-l/2373.pdf).

To assess soil quality, bioassays can be useful tools in monitoring the effects of soil pollution. Contaminants cause acute and chronic effects depending on species, time of exposure and end-points measured. These effects include lethality, growth, biomass, respiration rate, enzyme activity, photosynthesis, reproduction, and behavioural effects.

4. TOXICITY TESTING WITH EARTHWORMS

4.1. Relevance of the earthworm as test species

Earthworms are relevant test-organisms in ecotoxicological tests because they are common in a wide range of soils, representing 60-80 % of the total soil animal/invertebrate biomass. Earthworms have intimate contact with the soil and are the base of many food webs. They are also known to accumulate large concentrations of metals into their tissues when exposed to contaminated soils. Earthworms play an essential role in maintaining the structure and fertility of soils; recycling nutrients, increasing aeration and drainage, and can constitute an important component of the diet of birds, reptiles or small mammals (Allen 2002). Thus, earthworms are useful biological indicators of pollutants in soil.

In soil ecotoxicology, acute and chronic standardized tests have been developed using soil dwelling invertebrates, such as earthworms (ISO 1998a; ISO 1998b), potworms (ISO 2004) and

collembolans (ISO 1999). Earthworm species chosen for the standards are *Eisenia fetida* and *Eisenia andrei,* while *Lumbricus* species have especially been used for research purposes.

Earthworm toxicity tests are currently used as a basis for international regulatory guidelines in EU risk assessment (Langdon *et al.* 2005). Earthworms have been important organisms in toxicity testing for over 20 years. However, there are also several problems (Lowe and Butt 2006). *E. fetida* is the most widely used test species. The use of *E. fetida* has been questioned because it is more tolerant than most earthworms to contaminants and it is not common in natural soils. Sometimes field-collected "wild" earthworms are used and their genetic background and local subspecies can remain unknown. There can be problems with extrapolation from laboratory to field scale. The soil's physical and chemical characteristics, the kinetics of bioaccumulation, storage and excretion, and the organism's tolerance affect the bioavailability of arsenics (Langdon *et al.* 2005).

4.2. Interactions between soil, earthworms and arsenic compounds

Earthworms can be used as indicators of arsenic soil contamination. Earthworms are known to inhabit arsenic-rich soils. Due to their intimate contact with the soil (ingestion and dermal contact) they accumulate arsenic compounds present in soils (Fig. 8). Earthworms play an important role in enhancing organic matter turnover. Organic matter in soils is derived from debris of plants and animal residues. Earthworms have a high capacity for accumulating toxic elements through ingestion and dermal contact. Some populations found at these sites have exhibited resistance to arsenic-toxicity in toxicity tests (Langdon *et al.* 1999; 2001a and b; 2003). The mechanisms of resistance are not clearly understood. However, the extent of accumulation and the toxicity of arsenic compounds are dependent on the properties of the soil (4.1.1), of the earthworms (4.1.2) and of the arsenic compounds (4.1.3), and the processes between them.

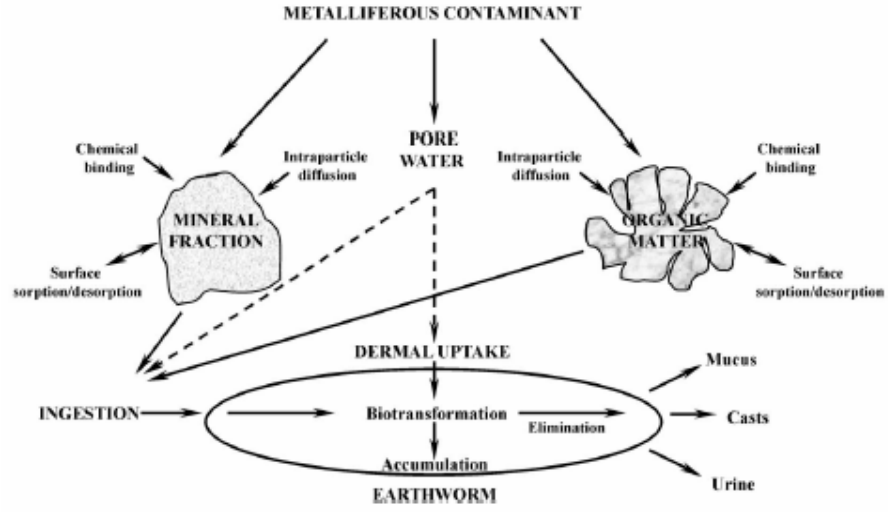

Figure 8. Processes between earthworms, soil and arsenic (= metalliferous contaminant) (Langdon *et al.* 2003).

4.2.1. Edaphic factors

The soil environment (edaphic factors) may affect the toxicity of arsenic compounds to earthworms. However, there has been little investigation of soil conditions and toxicity testing. There is a small amount of information on soil quality (Table 4). Meharg *et al.* (1998) studied how edaphic factors, such as soil pH, soil organic matter content, soil depth and exposure time affected the toxicity of arsenate in *Lumbricus terrestris*. They exposed earthworms to a range of arsenic concentration in soils at differing depths of a forest soil profile.

4.2.1.1. Soil pH and soil organic matter

Meharg *et al.* (1998) studied the effect of soil pH and soil organic matter on toxicity. Soil pH increased by 1.5 units between the top and bottom soil layer, whereas organic matter content decreased by 14.4-fold down the soil profile. After a 4-day exposure earthworms in the top and bottom layer of the soil lost 10-15 % weight. In contrast, earthworms living in the intermediate layers increased in weight by 5 to 20 %.

4.2.1.2. Soil depth

When Meharg *et al.* (1998) studied the effect of soil depth on toxicity after a 4 d exposure, the toxicity of arsenate increased with depth down the soil profile, with the 4-d LC50 decreasing from 300 to less than 100 mg/g at the extremes of the soil profile. (LC50 –value is the concentration estimated to reduce the survival of the test organisms by 50 % compared to the control.) These data suggested that the edaphic conditions, pH and organic matter, of the top and bottom soil may have been poor for the earthworms. Soil conditions affected sub-lethal and lethal effects. However, interpretation of toxicity results is complicated.

4.2.1.3. Exposure time

Meharg *et al.* (1998) studied how the length of exposure time affected the toxicity of arsenate to the worms (Fig. 9). In arsenate-dosed soil (0-500 mg As/kg), toxicity increased fourfold between 1 and 10 days. Exposure time was important to toxicity testing. Figure 10 describes the accumulation of arsenic in earthworm tissue over time (days) from soil contaminated with 40 µg g^{-1} dry weight (Meharg *et al.* 1998).

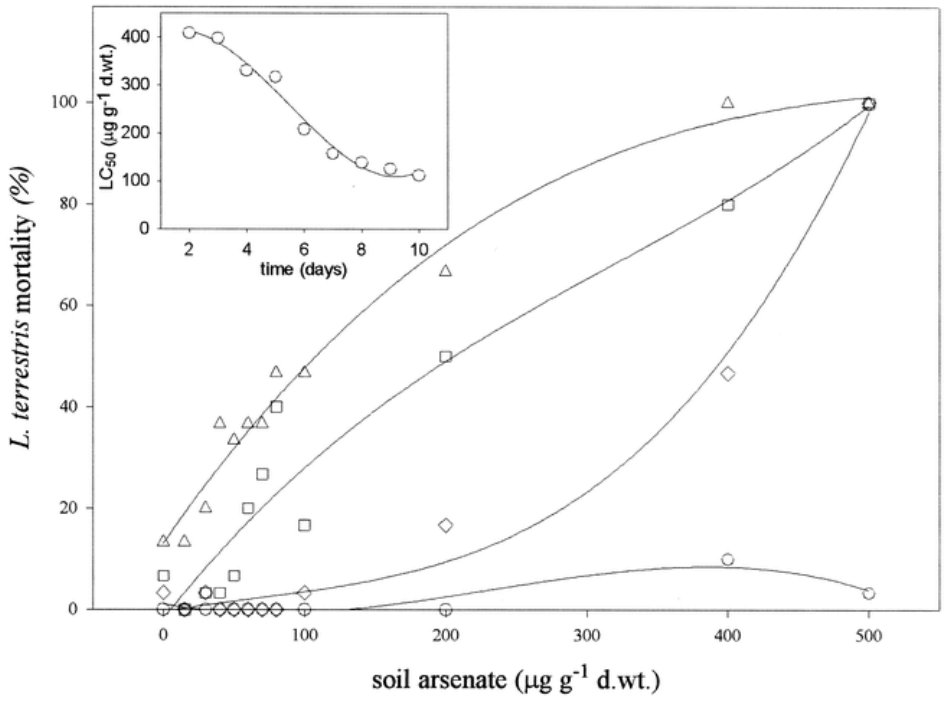

Figure 9. Earthworm mortality (percent) at 1 (circles), 3 (diamonds), 6 (squares), and 10 (triangles) d of exposure over a range of arsenate concentrations according to Meharg *et al.* (1998). The insert shows the concentration that causes 50% mortality calculated for each day of the experiment.

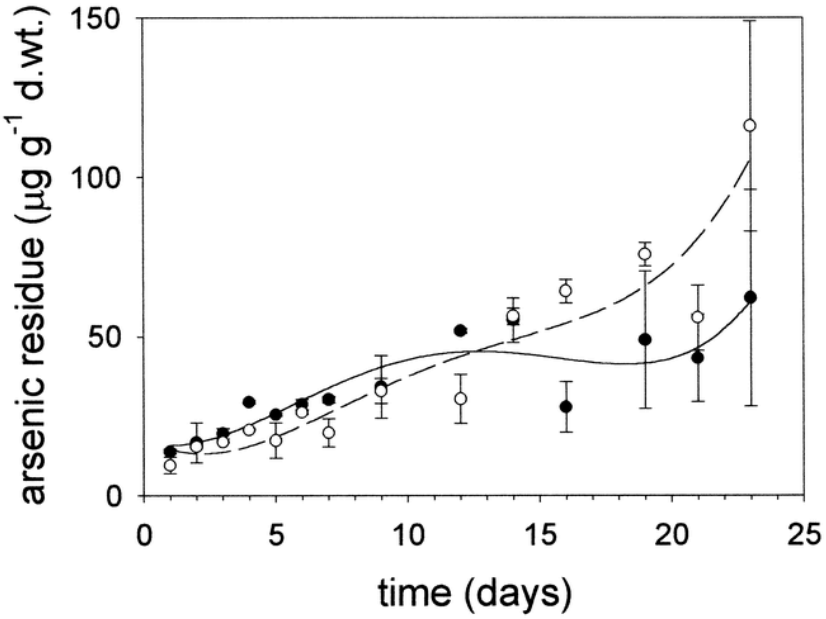

Figure 10. Accumulation of arsenic in earthworm tissue over time expressed as micrograms per gram earthworm dry weight from soil contaminated with 40 µg g^{-1} dry weight according to Meharg *et al.* (1998). The filled symbols represent undepurated worms, and empty symbols represent depurated worms.

4.3. Biological factors of earthworm tests

4.3.1 Species' sensitivity

Earthworms have intimate contact with the soil. With chemoreceptors in the prostomium (part of the mouth) and sensory tubercles on their body surface, they can have a high sensitivity to chemicals in soil (Reinecke *et al.* 2002). Chemical sensitivity and mobility enables worms to avoid toxic arsenic compounds. Thus, soil avoidance tests can be useful. Soil avoidance tests conducted in the laboratory by Langdon *et al.* (2001), in which earthworms were given a choice of uncontaminated or arsenic-treated soil, showed that *Lumbircus rubellus* from mine spoil, containing high concentrations of arsenic (8000 mg/kg), only discriminated significantly against soil containing concentrations of sodium arsenate above 5000 mg/kg by moving into the uncontaminated soil. Below this concentration, the earthworms did not discriminate between clean and arsenic-treated soils.

Some populations found at the arsenic contaminated sites have exhibited resistance to arsenic-toxicity in toxicity tests. Langdon (*et al.*, 2001) found populations of *L. rubellus* resistant to arsenate- and copper-toxicity present in mine spoil containing up to 8000 mg As /kg and 750 mg Cu/kg. In contrast, Yeates *et al.* (1994) found no earthworms in soils contaminated by arsenic derived from timber preservatives at concentrations of 400 and 800 mg As/kg, and few earthworms at 100 mg As/kg. The soils that Yeates *et al.* (1994) examined contained other contaminants in addition to arsenic. It is possible that the results of possible antagonistic or synergistic effects are important in determining toxicity to earthworms in soils containing several contaminants.

Differences in behaviour may render some species more susceptible to toxins in the soil than others. Three ecological types of earthworms can be identified:

1. litter dwelling epigeic species, for example, *Lumbricus rubellus*;
2. mineral soil dwelling endogeic mineral soil dwelling species, for example, *Aporrectodea caliginosa*;
3. deep vertical burrowing, litter-feeding anecic species, for example, *Lumbricus terrestris*.

Eisenia fetida is an ultra epigeic species (living almost entirely in organic matter) currently used as the standard earthworm in terrestrial ecotoxicology tests in ISO 11268 (ISO 1998a; ISO 1998b). The OECD acute earthworm toxicity test (OECD 1984) uses *E. fetida/ andrei* earthworms as biological monitors for testing the effects of contaminants on soil biota. The two species are very similar, both in their physiology and their mode of life. However, many European species of earthworm behave differently than *E. fetida/ andrei* (Langdon 2001, 2003 and 2005; Johnson *et al.* 2002; Piarce *et al.* 2002; Arnold *et al.* 2003).

4.3.2 Accumulation

Earthworms can accumulate arsenic compounds from arsenic contaminated sites (Table 5). Fischer and Koszorus (1992) found considerable accumulation of arsenic (bioconcentration factor 10.30-18.10) in *E. fetida* exposed to sub-lethal concentrations of arsenate. Some earthworm species have been reported not to accumulate arsenic (bioconcentration factor 0.1-0.67, Table 5). There is no direct correlation between soil arsenic concentration and tissue arsenic concentration. This may depend on different modes of life between earthworm species, exposure time, different routes and analytical methods employed (Langdon *et al.* 2003; Langdon *et al.* 2005).

4.3.3 Lethal and sub-lethal effects

Toxicity in earthworms can be measured in two ways: mortality and sub-lethal effects. Toxicity may affect survival, growth, reproduction (cocoon production and viability), behaviour (soil selection and perhaps level of activity), metabolism, pigmentation and composition of the earthworm communities. Fischer and Koszorus (1992) recorded that *E. fetida* had a 56 day LC50 of 100 mg/kg for exposure to arsenate. Meharg *et al.* (1998) demonstrated an LC50 of 100 mg/kg arsenate for an 8-day exposure of *L. terrestris*, and 400 mg/kg for 2-day exposure. Linder *et al.* (1994) found no difference in survivorship of *E. fetida* between contaminated (mean 58 mg As/kg) and control soils (<11 mg As/kg). In the study by Linder *et al.* (1994), several of the soils had total arsenic residues of over 100 mg/kg. Earthworms are able to sequester arsenic species in their tissues in less toxic forms than arsenate when the accumulation is over a long time period (Fig. 9). Piearce *et al.* (2002) found yellow earthworms, *Lumbricus rubellus*, in at mine from the 19th century. These worms had a distinctive yellow pigmentation associated with arsenic- and copper-tolerance.

4.4. Arsenic detoxification

The mechanisms of arsenic resistance in earthworms are not clearly understood and may be adaptive, for example genetically based, or represent physiological acclimation. Earthworms have proteins, metallothioneins, which bind metals. Metallothioneins have been used as biomarkers for metal contamination and may play a role in the detoxification of arsenic from contaminated earthworms (Fig. 9) (Langdon *et al.* 2002; 2003; 2005).

Table 4. Investigations of As-contaminated soils and earthworms.

Species	Soil	Soil As, mg/kg	Conclusions	Reference
E. fetida	As-contaminated site	23-50	Considerable bioconcentration in worms exposed to sub-lethal concentrations of arsenic. Juvenile mass and production of adult cocoon were decreased significantly by sub-lethal concentrations of arsenic.	Fischer and Koszorus 1992
L. terrestris	Timber preservative CCA	12-790	Contamination affected soil biological activity; basal soil respiration etc. Sulphatase activity was the most sensitive to high contamination. *L. rubellus* was absent in plots with medium and high contamination.	Yeates *et al.* 1994
L. terrestris	Mixed beech-pine forest soil	74	Toxicity increased 4-fold between 2 and 10 d (exposure time). Edaphic factors (soil pH, organic matter, depth) affected toxicity.	Meharg *et al.* 1998
Lumbricidae	As-contaminated sites (uncontaminated sites)	50	No strict correlation between tissue and soil As concentration.	Geiszinger *et al.* 1998
L. rubellus	Copper/arsenic mine	50 000	Populations at this site are tolerant to very high concentrations of As.	Langdon *et al.* 1999
L. rubellus	Copper/arsenic mine	50 000	Clear difference in the LC50s of tolerant and non-tolerant populations (1510 and 96 mg As/kg, respectively).	Langdon *et al.* 2001a
L. rubellus	Mine	9845	Earthworms showed high tolerance to As-toxicity and had a striking yellow coloration.	Pierce *et al.* 2002
L. rubellus	Copper/arsenic mine	50 000	As speciation in the arsenate-resistant worms: As was predominantly co-ordinated with sulphur in the form of a SH group (metallothionein complexation), also small amounts of arsenobetaine.	Langdon *et al.* 2002
L. rubellus	Mine spoil site	162-566	As speciation in the worms: As was predominantly as arsenobetaine, also as arsenate and arsenite. Hypothesis: arsenic induces the expression of metallothionein in earthworms and is sequestered by the metalloprotein in certain target cells and tissues.	Langdon *et al.* 2003 and Langdon *et al.* 2005

Table 5. Concentrations of total arsenic in earthworm tissue according to Langdon *et al.* 2003.

Species	Soil conc. mg/kg	Tissue conc. mg/kg	pH	Bioconcentration factor *)	Reference
Aporrectodea rosea	79.1	40	5.7	0.5	Yeates *et al.* 1994
E. fetida	87	902	-	10.30	Fischer and Koszorus 1992
E. fetida	23	418	-	18.10	Fischer and Koszorus 1992
E. fetida	50	643	-	12.80	Fischer and Koszorus 1992
L, rubellus	494	230	5.1	0.46	Langdon *et al.* 1999
L. rubellus	8930	620	7.1	0.069	Langdon *et al.* 2001 a, b
L. rubellus	79.1	41.4	-	0.52	Yates *et al.* 1994
Lumbricidae	79.7	17.9	5.6	0.22	Geiszinger *et al.* 1998
Lumbricidae	5.0	3.2	6.5	0.64	Geiszinger *et al.* 1998
Lumbricidae	45.7	8.2	5.8	0.18	Geiszinger *et al.* 1998
Lumbricidae	48.8	4.8	7.7	0.10	Geiszinger *et al.* 1998
L. terrestris	73.9	50	-	0.67	Meharg *et al.* 1998

*) Bioconcentration factor = earthworm tissue concentration/soil concentration.

4.5. Speciation of arsenic in earthworms

The chemical forms and oxidation states of arsenic are important in determining the level of toxicity in earthworms. The toxicity of arsenic also depends on other factors such as the physical state of the arsenic, particle size of the matrix or content of solution, the rates of uptake and elimination from cells and the pre-existing state of the organism (Mandal and Suzuki, 2002). The soluble inorganic arsenicals are more toxic than organic species. As(III) is more toxic than As(V).

There has been a few physiological studies on toxicity and metabolisms of arsenic in earthworm species. Speciation of arsenic in earthworm tissues appears mainly to be in inorganic forms (As (III) and As (V)) and may also differ between species (Fig. 9). Earthworms are able to sequester arsenic species in their tissues in less toxic forms than arsenate (Langdon *et al.* 2003; Langdon *et al.* 2005).

Differences in depth in the soil and feeding behaviour may have produced differences in routes of arsenic uptake, speciation of the contaminants and toxicity tolerances. Langdon *et al.* (2002) studied arsenic speciation in arsenate-resistant *L. rubellus* and non-resistant *L. rubellus* from an uncontaminated site. Arsenic was predominantly co-ordinated with sulphur in the form of a SH group, suggesting metallothionein complexation. Another arsenic species was also present in field samples: As (V)-O (representing up to 30 % of the body wall As and 45 % of the whole earthworm As burden). The proportion of arsenate to sulphur-bound species varies within specific earthworm tissues (Langdon *et al.* 2005).

5. TOXICITY TESTING WITH TERRESTRIAL PLANTS

Plants are the primary producers, supporting all other life forms. The role of plants in soil development, stabilization, and nutrient cycling is important. The plant tests have been adopted to evaluate single chemical and mixed chemical effects. More recently, the plant tests have been used to evaluate soil contamination.

Higher plants provide valuable assay systems for screening and monitoring environmental pollutants. Plants transfer metal to higher trophic forms and should be considered when conducting an environmental assessment (Wang *et al.* 1997). The advantages possessed by higher plant assays, which are inexpensive and easy to handle, make them ideal for use in soil risk assessment.

5.1. Arsenic uptake in plants

The transfer of arsenic from soil to plant (Table 3) is very low for most common plant species. This is probably due to: i) the restricted uptake by plant roots, ii) the limited translocation of arsenic from root to shoot, iii) arsenic phytotoxicity even at low concentrations in plant tissues, and iv) the low bioavailability of arsenic in soil (Wang *et al.*, 2002). However, some plants have elevated tolerance to arsenic and they can accumulate high amounts of arsenic (Ma et al., 2001). If arsenic is taken up by plants, then it is transferred to the food chain (Table 3).

The amounts of arsenic absorbed by a plant (Fig. 11) depends on:

- the concentrations of As in the soil
- the bioavailability of As in the soil
- the speciation of As in the soil
- dissolved organic matter
- soil pH
- soil characteristics like clay, oxides and cation exchange capacity
- plant species
- the amount of root produced, etc. (Kalbitz &Wenrich, 1998).

5.2. Arsenic toxicity to plants

5.2.1. Concentration and speciation in plants

Arsenic concentrations in terrestrial plants are usually less than 10 mg kg^{-1} (Matschullat, 2000). Several plants contain arsenic in the following order: cabbage (0.020 – 0.050 mg kg^{-1}) < carrots (0.040 – 0.080) < grass (0.020 – 0.160) < potatoes (0.020 – 0.200) < lettuce (0.020 – 0.250) < mosses and lichens (0.26) < ferns (1.3) (Matschullat, 2000). In Finland, the typical concentrations in potatoes are below <0.01 mg kg^{-1} and in carrots from less than 10 mg/kg to 60 mg/kg (ref in Mäkelä-Kurtto *et al*., 2007).

The phytotoxicity of arsenic is affected by the chemical form in which it occurs in the soil and concentration; water-soluble form being more phytotoxic than other firmly bound forms. Arsenite, As(III) is more phytotoxic than arsenate, As(V) and both are much more phytotoxic than monosodium methane arsenic acid (MSMA).

Plants take up (Fig. 11) arsenic as arsenite and arsenate, the major forms of arsenic, which is greatly influenced by soil texture and competing phosphates. The concentration tolerated by plants is 1-50 mg As/kg soil. Low levels of phosphates displace arsenic from soil particles to increase uptake and phytotoxicity, while larger amounts of phosphates compete with arsenic at root surfaces to decrease uptake and phytotoxicity.

Pickering *et al.* (2000) studied the biochemical fate of arsenic in Indian mustard (*Brassica juncea*). After arsenate uptake by the roots, possibly via the phosphate transport mechanism, a small fraction is exported to the shoot via the xylem as arsenic oxyanions (arsenate and arsenite). Once in the shoot, the arsenic is stored as an As(III)–tris thiolate complex. The majority of the arsenic remains in the root as an As(III)–tris thiolate complex, which is indistinguishable from that found in the shoot and from As(III)–tris glutathione.

5.2.2. Phytotoxic effects

Arsenic is a nonessential element for plants. Inorganic arsenic is highly phytotoxic. Arsenic in inorganic and organic forms used previously as pesticides, plant defoliants, and herbicides accumulate in soils and in plants. An average toxicity threshold of 40 mg/kg was established for crop plants (Sheppard *et al*., 1992). The chemical behavior of arsenic is largely similar to that of phosphorus in soils. In all plant species tested so far, arsenate is taken up via the phosphate transport systems. Excessive concentrations of arsenic result in phytotoxicity:

- Inorganic As inhibits enzyme activity and trivalent inorganic arsenic reacts with the sulphydryl groups (-SH) of proteins affecting many enzymes.
- Due to its chemical similarity to phosphorus, arsenic participates in many cell reactions. Arsenic replaces phosphorus in the phosphate groups of DNA. Specific organo-arsenical compounds have been found in some organisms.
- Arsenites and arsenates (the reactions with sulphydryl groups and phosphorus) interfere with physiological and biochemical processes which constitute plant growth in a number of ways.
- As competes with phosphorus uptake of plants and causes P-deficiency resulting in the appearance of dark red leaves. Organo-arsenicals can apparently be metabolized. The carbon–arsenic bond is apparently stable in plants but is rapidly broken down in soils.
- Inhibition of physiological and biochemical processes by As result in reduction in morphological characters and economic yield of agricultural and horticultural crops. Major characteristics affected are tillers (in cereals), plant height, leaf number and area, pod number and length (in legumes), and dry matter production.

5.2.3. Phytotoxicity studies

Studies on toxicity of metals on native plants in field conditions are limited. Usually, the effects on plants are examined in laboratory conditions, which differ from field conditions. Much research has been focused on the effects of metals on food plant production and, until recently, rather less on trace metal cycling in natural ecosystems.

To understand the effects of toxic metals on soil–plant systems, studies on a number of aspects are required. These aspects include characteristics of the toxic metal and soil, mechanisms of metal on plant species, targets of action, etc. (Fig. 11).

Root-elongation test is the most common plant test for heavy metal toxicity in soils. In addition, various life history parameters have been used, such as germination, seedling growth, plant height, leaf number and area, pod number and length (in legumes), biomass production, dry matter production, reproduction. Physiological parameters have also been developed for pollutants that are not specific (responding all kinds of pollutants) or sometimes specific for a particular pollutant (e.g., phytochelatins as heavy metal-binding peptides). A very specific effect is the phytotoxic effect on plants due to inactivation of photosynthesis by heavy metals. There is no single plant test that adequately detects the types of toxicity induced by complex chemical mixtures and by all chemical compounds (Wang *et al.*, 1997).

There are several hundreds of reports in the literature concerning metal phytotoxicity laboratory tests and field experiments. However, interpretation of the results is problematic due to following aspects:
- soil properties influence the rates at which metals transfer to plants;
- phytotoxicity differ with plant species;
- roots may prevent translocation to the leaves;
- no chemical or toxicant interactions are taken into account; and
- the large number of environmental variables (chemical form of metal, soil type, pH, organic matter, plant species, associated microbial species, etc.) restrict our ability to interpret information
- changes in foliar chemistry may be influenced by other environmental factors such as water availability, pH, redox or salinity (Wang *et al.* 1997).

5.2.4. Genotoxicity of arsenic

Among soil pollutants, particular attention should be paid to soil mutagens. Soil pollution by heavy metals has increased because of air emissions, mainly from industrial sources. Genotoxic effects could, in part, be responsible for metal phytotoxicity.

Plant genotoxicity due to heavy metals has been known and documented for a long time (Wang *et al*. 1997). A number of assays have been developed which use higher plants for measuring mutagenic effects of contaminated soils. Plant mutagenity assays require less extensive equipment, materials and personnel than most other genotoxicity tests, which is a potential advantage. A Tradescantia micronuclei test was found to be the most sensitive plant genotoxicity test.

Arsenic can, when present in excess or under certain conditions, produce errors in the genetic information system (Patra *et al*. 2004). Arsenic is a weak mutagen and it cannot induce directly gene mutations. However, arsenic is a potent comutagen, an agent that will enhance the mutagenicity. Inhibition of enzymes involved in DNA repair by arsenic may be responsible for the DNA damage. Arsenic is also a clastogen that can cause microscopically visible damages or changes to chromosomes (e.g. breaks in chromosomes, change in chromosome number). Most metals are clastogenic to higher plants, *in vivo*, at certain concentrations and durations of exposure. Effects of metallic salts are related directly to the dosage and duration of exposure.

5.3. Arsenic tolerance

5.3.1 Metal resistance systems in plants

Plants growing on arsenic contaminated sites develop some degree of tolerance to metal toxicity. Since all plants contain at least some metals, they cannot exclude all toxic elements. Plants restrict their uptake or translocation.

Tolerance to arsenic can be achieved by avoiding the metal stress, by tolerating it or both.

Avoidance is the most common mechanism of plant adaptation to arsenic toxicity. It depends on various kinds of reduced metal uptake:

- by deposition in cell wall components; and
- by chelate secretion.

Tolerance to metal stress relies on plant capacity to detoxify metals having entered cells. The mechanisms for metal tolerance are:

- metal sequestration by specially produced organic compounds;
- compartmentalization in certain cell compartments;
- metal ion efflux;
- organic ligand exudation.

Inside cells, proteins such as ferritins, metallothioneins and phytochelatins and related peptides participate in excess metal storage and detoxification.

- Ferritins are a class of multimeric iron-storage proteins able to sequester several thousand iron atoms per molecule.
- Metallothioneins are small proteins that sequester excess amounts of metal. Their synthesis is activated by metal ions. Metallothioneins are gene-encoded, low molecular weight, cysteine-rich polypeptides
- Phytochelatin are specific thiol-rich proteins derived from glutathione.

When these systems are overloaded, oxidative stress defence mechanisms are activated. Arsenic triggers tissue and developmental stage specific defense responses of antioxidants (superoxide dismutase and catalase enzymes) and detoxification related genes (glutathione *S*-transferase) in maize. In several cases, plant survival has been related to tolerance to arsenic.

5.3.2. Arsenic accumulating plants

An important arsenic hyperaccumulating plant is *Pteris vittata,* Chinese brake fern (Ma *et al.* 2001). Metal hyperaccumulators can achieve metal concentrations > 1g As/kg (Francesconi *et al.* 2002). *Pteris vittata* produces large biomass and also accumulates arsenic up to 2 % of its biomass. Also, other ferns, such as *Pteris cretica, Pteris longifolia, Pteris umbrosa, Pteris calomalanos* accumulate arsenic (Zhao *et al.* 200; Francesconi *et al.* 2002; Li *et al.* 2005). Arsenic accumulating ferns are good phytoremediators of arsenic polluted soils.

The ability of hyperaccumulators to withstand the very high concentrations of arsenic in soil suggests that they have a mechanism to detoxify the arsenic. Metal-tolerant plants have efficient mechanisms for the detoxification of accumulated metal including chelation (metallothioneins and phytochelatins), compartmentalization, biotransformation and cellular repair mechanisms (Li *et al.* 2005). Arsenic detoxification might also include methylation and biotransformation by microorganisms. Some bacteria enzymatically reduce arsenate to arsenite by an enzyme called Ars C, and the arsenite is then pumped out by the membrane protein by another enzyme, Ars B (Cai & Ma, 2003). None of these mechanisms were identified in the ferns.

Li *et al*. (2006) hypothesized that the high arsenic concentrations may affect chloroplasts in *Pteris vittata*: The addition of arsenic did not affect the chloroplast ultrastructure of young pinna, while most of the membrane systems of chloroplasts in mature pinna were severely damaged under high arsenic condition.

The amount tolerated by plants varies from 1 to 30 mg As/kg soil, but plant species differ in this respect. Toxicity of arsenic depends on its chemical form and soil quality. Arsenic is easily available from soils with low ion-exchange capacity and with low colloid material concentrations. High clay, organic substance, Fe, Ca, P and N content in the soil decreases arsenic availability. The most essential factors affecting plant growth and arsenic accumulation are pH and phosphate. Phosphate and arsenate are chemical analogues and interaction between them needs special attention. pH is an important factor that influences the chemistry of arsenic and phosphorus. Phosphate availability in soils depends on the solubility of minerals (calcium phosphate, aluminium phosphate and iron phosphate) and maximum availability occurs when pH is between 6.5 and 7.5. Arsenic species varies with both pH and redox potential. Understanding the factors regulating the growth of hyperaccumulating plants are important (Tu & Ma 2003 and 2005; Baroni *et al.* 2004; Cao *et al.* 2003; Fayiga *et al.* 2004).

Speciation of arsenic in the accumulating plant can provide important information about the mechanisms of arsenic accumulation. According to Chang *et al.* (2002), arsenic in *Pteris vittata* was predominantly inorganic. The authors concluded that the fern uptakes arsenic as arsenate, which is converted to arsenite. The mechanisms of arsenic uptake and transformation by this plant are not known.

Plant characteristics • Plant species • Genetic material • Roots, form and biomass • Developmental stage of the entity affected • Residence time in the plant under study • Distribution of dose over time • Route of exposure (leaf, shoot and root)		**Biological effects of toxic metal action** • Bioavailability • Acute and chronic effects • Cumulative effects • Reproductive functions • Respiratory functions • Photosynthetic processes • Increase/decrease in populations
Soil properties • Soil type: sand, clay, peat etc • Soil mineralogy • Bulk density • Particle density • Particle size distribution • Soil moisture • Soil consistence • Soil cation exchange capacity • Soil pH • Redox potential • Soil fertility (N, P, K) • Phosphate concentration • The presence of other substances and toxicants • Rock fragments • Carbonates		**Characteristics of the toxic metal in soil** • Concentration • Chemical forms • Adsorption on clay, organic matter or oxyhydroxides • Complexes with organic and inorganic ligands • Interactions with other elements • Biologically occluded • Oxide occluded

Figure 11. The effects of toxic metals on soil – plant systems, studies on a number of aspects are required.

The use of genetic engineering to modify plants for arsenic uptake and transport may enhance the efficiency of phytoremediation, for example, by introducing arsenic chelator, arsenic transporter, metallothionein and phytochelatin genes into plants (Alkorta *et al.* 2004; Eapen & D'Souza 2005).

Some arsenic accumulating plants growing in old mines have been useful for biogeochemical exploration of arsenic (Pratas et al. 2005). These plants have arsenic tolerance and detoxification systems for polluted sites. Arsenic accumulation was found in the needles of *Pinus pinaste*, *Calluna vulgaris*, *Chamaespartium tridentatum* (Pratas *et al.* 2005). All *Andropogon scoparius* plants from a mine site in the USA possessed tolerance to arsenate and in the UK, tolerance in arsenic-toxic mine spoil *Agrostis* plants was specific to water-soluble arsenate. Only those plants found on soils with more than 15000 mg As/kg tolerated 25 g As/mL. Arsenic is one of the elements that have been successfully used to identify ore deposits through analysis from suitable tolerant "indicator" plant species. Porter and Peterson (1975) reported the presence of 6640 mg/kg arsenic levels in the herb *Jasione montana,* 4130 mg/kg in heather *Calluna vulgaris* and 3470 mg/kg in the grass *Agrostis tenuis* in old arsenic mine sites in Cornwall and Devon in England.

PART B - ECOTOXICITY TESTING WITH THE RAMAS SOIL SAMPLES

6. SAMPLES

Nineteen soil samples were collected in 2005 from an old wood preservation area, a mine tailing area and from areas where natural background concentrations of arsenic are relatively high. The top soil layer, approximately 5 cm, was removed and samples were collected as composite samples from 5 – 30 cm depths (contaminated sites). In the laboratory, samples were sieved (< 4mm), mixed thoroughly, and divided into sub-samples for storage at -20 °C. Clayey samples were left to dry overnight at room temperature and crushed in a mortar before sieving.

Chemical concentrations of metals and arsenic were determined by ICP-OES techniques (Geolaboratory, Geological Survey of Finland). Two extraction methods were used before chemical analyses: 1 mol/L ammonium acetate-EDTA pH 4.5 extraction and aqua regia extraction. Ammonium acetate –EDTA is often considered as the bioavailable fraction of metals in soil (Ernst, 1996). All samples were analyzed for basic soil properties: dry mass, pH, water holding capacity (WHC) and conductivity. Sample codes and the physico-chemical properties are given in Table 6, chemical concentration of As and metals are shown in Table 7.

Table 6. Soil samples and their physico-chemical properties.

Municipality	Sample ID	Depth cm	pH	Conductivity mS/m	Dry matter %	WHC %	Soil type
Natural soils							
Orivesi	**M1**	20-50	5.6	5.0	79	68	Clay
Orivesi	**M2**	15-50	5.9	2.5	82	68	Clay
Orivesi	**M3**	18-55	5.4	6.1	77	82	Clay
Pirkkala	**M4**	190	6.0	2.2	87	28	Till
Pirkkala	**M5**	230	5.8	2.3	85	Nd	Till
Hämeenkyrö	**M6**	3-80	5.4	1.5	92	48	Fine sand
Ylöjärvi	**M7**	3-70	4.9	3.5	79	Nd	Fine sand
Contaminated soil, wood preservation							
Ruovesi	**R1.3**	5-30	6.9	1.9	94	nd	Fine sand
Ruovesi	**R2.1**	5-30	5.8	1.5	95	Nd	Fine sand
Ruovesi	**R2.4**	5-30	6.3	1.5	96	40	Fine sand
Ruovesi	**R3.2**	5-30	5.6	2.0	94	Nd	Fine sand
Ruovesi	**R4.4**	5-30	6.5	1.6	94	38	Fine sand
Ruovesi	**R5.3**	5-30	5.6	2.4	91	Nd	Fine sand
Ruovesi	**R5.5**	5-30	5.6	2.6	93	42	Fine sand
Contaminated soil material, mine tailing							
Ylöjärvi	**L1**	5-30	5.4	26.5	98	61	Mine tailing
Ylöjärvi	**L2**	5-30	4.9	14.5	98	63	Mine tailing
Ylöjärvi	**L3**	5-30	5.5	1.4	100	58	Mine tailing
Ylöjärvi	**L4**	5-30	5.8	6.5	94	75	Mine tailing
Ylöjärvi	**L5**	5-30	6.0	0.7	100	53	Mine tailing

7. TEST METHODS

The principle aim of using toxicity tests in the RAMAS project was to produce data for the ecological risk assessment. Expect for a few published results (Turpeinen *et al*. 1999; Schultz *et al*. 2004), there is no data on ecotoxicologal effects on Finnish field soils and on arsenic in particular. Therefore, common terrestrial ecotoxicity methods were chosen to assess the effects on organisms in the presence of the samples collected in field. There was a preference for standardized procedures, but for practical reasons some modifications were unavoidable. To determine the effects on critical events in the lifecycle of the organisms, germination tests were applied to assess the plant's toxicity, and survival and reproduction of soil invertebrates were determined. Since the duration of a soil invertebrate reproduction test is several weeks, it was not possible, during this project, to test all the samples with all tests, but only a selected set of samples. In addition to the tests on solid samples, we also used two aquatic tests: duckweed growth inhibition and an enzymatic *in vitro* test. For use as a positive control, OECD artificial soil was spiked with sodium arsenate, and tested along with the project samples. Spiked samples were either tested after stabilization for one day or after ageing for one year.

All contaminants, not only arsenic, will affect on the organisms when they are exposed to field samples. Therefore, it was important to differentiate the cause and effect relationships. The arsenic specific effects on toxicity were determined using an elaboration model based on covariance analysis parameters (SPSS software, version 11).

Seed germination tests were performed with two different plant species: ryegrass (*Lolium multiflorum*), and lettuce (L*actuca sativa*). The lettuce test followed the ISO 17126 method and the ryegrass method was a modification of the ISO standard 11269-2. Test conditions in test chambers were the same for both species: temperature 20 °C, light 4300 ± 430 lux , light cycle16 h light/8 h dark, and humidity 80 %. At the beginning of the test, the test vessels were kept in the dark for the first 48 hours, followed by a light cycle until the end of test period. Clean natural sand was used as the control soil. Dry seeds were put on top of the test soil, and 90 g of the cover sand was spread out evenly on the top of the seeds. The material was wetted with de-ionized water. At the end of the test, the number of the germinated seeds was recorded. Results were calculated as percentage inhibition and the statistical significance was calculated with the Student's t-test (SPSS software, version 11.0).

Duckweed (*Lemna minor*) plants are small free floating plants that take all nutrients directly from the water. This fact allows for the testing of phytoavailability of soil contaminants via water. We tested the effects on duckweed growth by exposing the plants to a medium containing the solid sample. Fifty grams of samples were put on the bottom of test vessels and 100 ml of standard growth medium (ISO/FDIS 20079) was added into the vessels. The growth medium contained all nutrients necessary for normal growth. According to the standard's scope, the test was suitable for testing wastewater and water soluble chemicals. The present modification of the standard test measures the effects of easily leachable contaminants, because no shaking of the soil and solution was performed. Plants were grown at 20 ºC for 7 days and the number of the fronds and the frond area were measured as test parameters using imaging (Scanalyzer, LemnaTec GmbH, Germany). Plants were grown in glass vessels (diameter 80 mm) that were covered by a glass lid. In the control vessels, soil was replaced by clean natural sand. Growth in control and sample vessels was compared and the effects were calculated as the percentage inhibition of growth.

The Pot worm, *Enchytraeus albidus,* test was performed according to the ISO standard 16387, Annex B. In the acute part of the test, ten adult worms were added to glass vessels containing 30 g

of test material. After 3 weeks' incubation at 20 °C the adult worms were removed and the number of living worms was counted. The incubation continued for 3 additional weeks to assess the effects on reproduction. At the end of the test, juveniles were isolated from the test material by wet extraction, and the animals were stained and the numbers were counted. Samples were diluted with the artificial soil (OECD artificial soil, containing Sphagnum peat, kaolinite and crushed quartz, pH adjusted to 6,0 ± 0,5) to different test concentrations. Chemical concentrations of the contaminants served as the basis for selection of samples to be tested and to prepare the dilutions. The aim was to calculate the EC50 values for both mortality and reproduction using probit analysis (SPSS software, version 11).

Earthworm (*Eisenia fetida*) survival and reproduction tests were performed according to standards ISO 11268-1 and ISO 11268-2 with some modifications. The standard procedure was followed in other respects, but the number of animals was reduced from 10 to 6 and the number of replicates was three instead of four, and the test material per vessel was 200 g instead of the 500 g recommended in the standard. The vessels were incubated for 4 weeks to determine survival. Adult worms were removed, and incubation continued for 4 additional weeks. At the end of test, juvenile worms were isolated from the medium for counting. Nine samples out of the 19 samples in total were investigated either at a single concentration or multiple concentrations. The aim was to determine the EC50 values as in the pot worm test.

Bioavailability of arsenic and metals to earthworms was determined in connection with the earthworm toxicity tests. Chemical concentrations of arsenic and metal were determined for worms from those test vessels where no acute toxicity was detected, that is, all worms were alive. After 4 weeks of exposure to the samples, the adult animals were removed from the vessels, rinsed with water, wiped with soft paper and their fresh weights were recorded. Worms from two replicates out of four were pooled and analyzed for As and metals with gut contents and the other two replicates were put on wet filter paper to empty their gut. After depuration of 3 or 24 h, the animals were weighed again and deep-frozen in liquid nitrogen. Earthworm samples were lyophilized and tissues were homogenized (Planetary mill, Fritsch, Germany). The ICP-MS technique was used to analyze elements after microwave assisted nitric acid digestion of the dry tissue homogenates. Chemical analyses were performed at the SYKE laboratory.

The reverse electron transport (RET) test is an enzymatic *in vitro* test suitable for the testing of water soluble chemicals. The method is based on the measurement of absorbance change in a microplate format. Electron transport is a chain of reactions essential for energy production in the mitochondria of living cells. These reactions are ubiquitous among eukaryotic cells and hence, the effects in RET reactions should represent possible effects on a wide range of organisms. Since the Ret test is an *in vitro* test, it measures the effects in direct contact with chemical compounds, and hence, only serves to detect the potential toxicity to whole organisms. Solid samples were extracted with water (10 g sample + 10 ml water) prior to the RET test. The mixture was shaken in a rotary shaker for one hour, filtered and centrifuged to get a clear supernatant for the assay. Before the RET assay, the pH of the samples was adjusted with 0,1 mol/L NaOH to 7.5 ± 0.2. The assay is based on the use of sub-mitochondrial particles prepared from isolated beef heart mitochondria (Knobeloch *et al*. 1994; Read *et al.* 1998). The reaction mixture consisted of succinate, antimycin A, NAD+, ATP, sub-mitochondrial particles and sample dilution or water as a control, in HEPES buffer, pH 7.5. The reduction of NAD+ to NADH was measured kinetically at 340 nm in a microplate reader (iEMS, Ascent, Labsystems, Finland). Sample extracts were diluted with water in twofold serial dilutions to achieve assay concentrations from 78.3 to 0.038 % of the extracts. The enzyme activities from the sample dilutions and controls were compared to calculate the inhibition percentages. EC50 values were estimated from the regression curves of inhibition versus water extract concentration.

Table 7. Metal concentrations of soil samples after aqua regia digestion and ammonium acetate - EDTA extraction.

AQUA REGIA DIGESTION		Concentration, mg/kg								
	Sample ID	As	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
	Natural soils									
	M1	*3*	<0.5	16	41	14	29000	20	14	97
	M2	13	<0.5	25	57	23	41100	28	16	111
	M3	*6*	<0.5	27	59	27	42600	31	17	176
	M4	30	<0.5	7	45	24	29700	13	13	47
	M5	111	<0.5	13	56	39	34000	21	12	54
	M6	<10	<0.5	8	18	12	17600	8	7	37
	M7	<10	<0.5	11	32	21	25700	13	18	66
	Contaminated soil, wood preservation area									
	R1.3	421	1	4	228	183	7330	6	13	22
	R2.1	351	1	4	126	153	7770	6	13	23
	R2.4	261	1	4	128	144	7340	6	7	21
	R3.2	724	2	4	291	269	7750	6	12	25
	R4.4	1960	5	3	875	910	7420	7	24	32
	R5.3	4080	9	3	1990	1050	6880	7	24	19
	R5.5	50	<0.5	4	58	28	8080	6	7	19
	Contaminated soil material, mine tailing									
	L1	2380	5	20	30	125	118000	15	26	224
	L2	2070	4	12	32	70	121000	13	25	219
	L3	1060	2	7	33	32	123000	12	26	188
	L4	2340	4	16	33	120	117000	14	30	226
	L5	2280	4	9	31	39	95800	12	26	180
AMMONIUM ACETATE EDTA EXTRACTION	**Natural soils**									
	M1	<3	<0.1	1	0	<3	548	0	<2	1
	M2	<3	<0.1	2	0	<3	565	1	2	1
	M3	<3	<0.1	1	<0.3	<3	384	1	<2	<0.8
	M4	5	<0.1	1	<0.3	<3	253	0.2	<2	<0.8
	M5	5	<0.1	0,4	<0.3	<3	200	0,1	<2	<0.8
	M6	<3	<0.1	<0.3	<0.3	<3	100	<0.1	<2	<0.8
	M7	<3	<0.1	<0.3	<0.3	<3	459	0	2	<0.8
	Contaminated soil, wood preservation area									
	R1.3	30	<0.1	<0.3	5	55	18	<0.1	3	1
	R2.1	27	<0.1	<0.3	4	43	14	<0.1	<2	1
	R2.4	20	<0.1	<0.3	4	43	13	<0.1	2	1
	R3.2	46	0,1	<0.3	8	95	15	<0.1	3	3
	R4.4	137	0,3	<0.3	20	511	28	0,2	6	7
	R5.3	151	0,4	<0.3	17	546	32	0,2	4	3
	R5.5	<3	<0.1	<0.3	4	12	43	<0.1	<2	<0.8
	Contaminated soil material, mine tailing									
	L1	703	2	2	<0.3	29	1170	0.5	5	5
	L2	602	2	0,5	<0.3	18	1320	0.1	2	1
	L3	193	0.5	<0.3	<0.3	4	620	0.2	<2	<0.8
	L4	627	2	1	<0.3	23	1260	0.3	5	3
	L5	454	1	<0.3	<0.3	11	825	0.1	3	<0.8
	samples M1 and M3: As was determined by GFAAS method after aqua regia digestion									

8. RESULTS

Total arsenic concentrations in soil samples varied from 3 mg/kg in the natural soils to more than 4000 mg/kg in contaminated soils (Table 7). Some samples taken from an old wood preservation area contained high metal concentrations, especially Cr and Cu concentrations, while toxic heavy metals (Cd, Pb) in all samples occurred in either low or moderately low concentrations. The average fraction of arsenic extracted with ammonium acetate-EDTA solution was 7 % for CCA –soils and 25 % for mine tailing samples. The fraction extracted with this solution did not vary markedly within the sample soil type. From natural soils, ammonium acetate EDTA extracted concentrations were under detection limit (3 mg/kg) in all but two samples, M4 and M5.

Results of the germination tests showed that rye grass germination was not affected significantly by the samples (Table 8). Only in the presence of the CCA-soil was some inhibition recorded, which, was not statistically significant in the Student's t-test. Effects on lettuce germination were more evident. Statistically significant inhibition ($p < 0,05$) was seen in five samples (M3, M4, R2.4, R3.2 and R5.3). When OECD soil was spiked with sodium arsenate ($(Na_2HAsO_4 \times 7\ H_2O)$ and tested for lettuce germination, the EC50 value corresponded to 110 mg As/kg.

Table 8. Results of the ecotoxicological tests presented as percentage inhibition or EC50 –values.

	Seed germination		Soil invertebrate acute and reproduction toxicity				Aquatic test methods	
	Inhibition %		Enchytraeus		Eisenia			
Sample	Rye grass	Lettuce	Acute toxicity	Reproduction EC50 (%)	Mortality, %	Reproduction EC50 (%)	Lemna, growth inhibition, %	RET, EC50 (%)
M1	2	2	nt	23	67	EC<100	3	6
M2	7	15	nt	22	nd	nd	0	1
M3	9	48	nd	nd	nd	nd	0	18
M4	0	70	nt	62	100	EC<100	11	>80
M5	0	10	nt	55	0	75<EC<100	0	>80
M6	1	0	nt	54	0	EC<100	24	>80
M7	5	6	nd	nd	nd	nd	21	>80
R 1.3	9	3	nd	nd	nd	nd	73	19
R 2.1	14	18	nt	23	0	17	97	11
R 2.4	2	29	nt	42	3	EC<50	100	6
R 3.2	12	26	nd	nd	nd	nd	97	11
R 4.4	4	13	nt	19	0 *)	4	98	1
R 5.3	4	56	nd	nd	nd	nd	99	8
R 5.5	0	0	nt	36	0	<100	68	12
L 1	0	11	nt	40	42 *)	0<EC<15	83	69
L 2	6	0	nt	29	nd	nd	94	25
L 3	0	2	nt	25	nd	nd	34	>80
L 4	0	0	nd	nd	nd	nd	67	>80
L 5	2	3	nd	nd	nd	nd	49	>80

nd, not determined; nt, not toxic; *) sample soil concentration 50 %

Table 9. Arsenic and metal concentrations in control earthworms after 0, 3 or 24 hours' depuration time. Worms were incubated for 4 weeks. Concentrations in the worms taken from basic culture (BG) are also given. All values are the mean values (mg/kg dw) from 3 -10 replicates, one replicate containing at least 3 adult animals.

Depuration time	Concentration, mg/kg							
hours	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
0	1.2	0.8	0.6	0.9	6	0.4	8.1	59
3	1.7	1.1	0.9	0.9	7	0.4	6.1	68
24	0.7	0.5	0.3	0.3	6	0.4	0.9	67
BG, 24 h	1.1	0.6	0.2	0.3	8	0.5	0.4	65

Duckweed growth was also inhibited in the presence of the soil samples. The modified duckweed test resulted in an almost complete lack of growth (> 90 % inhibition) or markedly decreased growth (> 30 % inhibition) in the presence of the CCA-soils and mine tailing samples, while the natural soils had only a minor effect, if any at all (Table 8). Photographs taken at the end of test demonstrate clearly the adverse effects on the duckweed health (Appendix).

The enzyme test (RET) responded to the majority of the samples. According to this test, water extracts of the CCA –soil were very toxic, with the EC50 values being less than 20 % for all these samples. The natural soils and the mine tailing samples had varied effects, both clear toxicity and moderate effects were seen, and some samples did not inhibit the reaction at all (EC50 >80 %).

Acute toxicity to enchytraeids was not observed, while reproduction was markedly decreased in the presence of all the samples, which were analyzed. The EC50-values varied between 22 and 62 % (Table 8). In general, earthworms were more sensitive than enchytraeids, showing higher mortality and lower reproduction compared to enchytraeus tests results of the same samples.

Soil animal tests were performed on selected samples from each sample type group (natural/ CCA soil/mine tailing). The aim of the acute and reproduction tests was to determine the EC50 values for both end-points. This turned out to be difficult because the dose response curves were extremely steep in many cases.

Analyses of the arsenic and metal concentrations of the earthworm tissues were performed on the animals after 4 weeks exposure, i.e. at the end of the acute phase of the toxicity test. Earthworms with no visible signs of adverse effects were from sample soil concentrations where no acute toxicity had been recorded in any of the replicate vessels. Tissue concentrations in control worms exposed to OECD artificial soil and from the basic culture are given in Table 9.

Table 10. Mean concentrations of arsenic and metals in earthworm tissue exposed to the samples for 4 weeks.

				Earthworm tissue concentration, mg/kg dw							
Sample type	**Sample ID**	**Test soil conc, %**	**Depuration time, h**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Natural soil	**M1**	100	0	1	0.6	7	3	5	1.2	0.7	52
Natural soil	**M5**	100	0	22	0.2	3	5	7	2.0	0.8	34
Natural soil	**M6**	100	0	2	0.7	3	5	9	2.0	1.2	59
Mine tailing	**L1**	33	0	170	0.3	5	1	8	0.6	1.1	46
CCA-soil	**R2.1**	100	0	60	0.2	2	7	29	1.0	0.7	46
CCA-soil	**R2.4**	100	0	370	0.8	1	9	48	0.7	0.7	72
CCA-soil	**R4.4**	50	0	325	0.7	1	64	130	1.0	5.4	67
CCA-soil	**R5.5**	100	0	29	0.3	1	2	8	0.6	0.5	43
Natural soil	**M1**	100	3	1	0.8	9	3	6	1.5	0.9	55
Natural soil	**M6**	100	3	2	0.8	4	5	10	2.1	1.2	61
CCA-soil	**R2.4**	100	3	335	0.7	1	15	53	0.8	0.8	70
CCA-soil	**R4.4**	50	3	325	0.7	1	62	125	0.8	4.5	73
Natural soil	**M5**	100	24	23	0.6	4	2	8	1.1	0.6	60
Mine tailing	**L1**	33	24	215	0.4	8	0.5	10	0.5	0.6	62
CCA-soil	**R2.1**	100	24	105	0.3	1	11	53	1.0	0.9	64
CCA-soil	**R4.4**	20	24	260	0.2	0.3	5	30	0.3	1.1	60
CCA-soil	**R5.5**	100	24	40	0.4	1	1	9	0.4	0.3	58

Maximum As concentrations measured from the earthworms after combining all the animals from one replicate vessel, with the gut contents, were approximately 400, 250, 400 and 40 mg/kg, for spiked, mine tailing, CCA, and naturally As-rich soils, respectively. Total As concentrations in soils varied for spiked soil from 3 – 100, mine tailings from 1000 – 2400, CCA soils 50 – 4100 and natural soils 3 – 111 mg As/kg. Arsenic concentration in worm tissue increased with the soil As concentrations as shown in Fig A.

Acute toxicity was not observed when sodium arsenate (40 – 200 mg As/kg, freshly prepared) was added to the artificial soil and used as the test medium, while reproduction was totally inhibited at the lowest concentration.

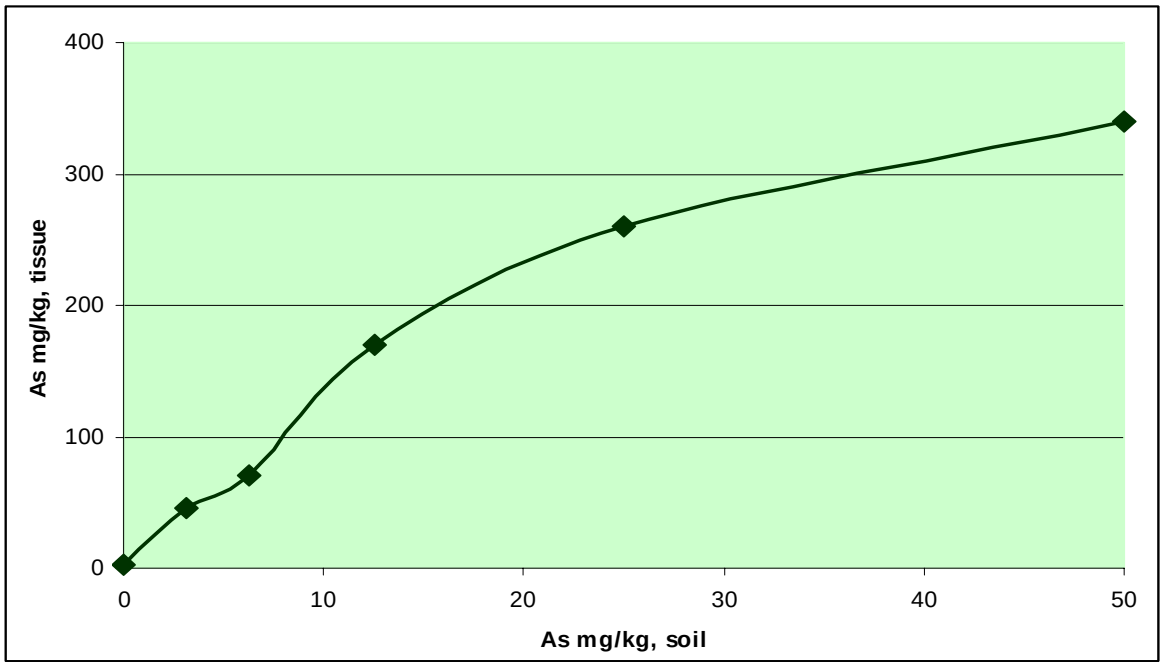

Figure. 12. Arsenic accumulation in earthworm tissue as the function of soil As concentration. The animals were exposed to As-spiked artificial soil for 4 weeks, depuration time 3 h on wet filter paper.

Figure 13. Arsenic concentrations (mg/kg) in earthworm tissue and test soils. Earthworms were analyzed after exposure to natural soil (M5), mine tailing (L1) and CCA -contaminated soils (R2.1 and R5.5) for 4 weeks. Results of depurated and non-depurated animals are combined. Microwave assisted nitric acid digestion and ICP-MS techniques were used for tissue analyses. Soil concentrations were determined by ICP- OES after aqua regia digestion or ammonium acetate - EDTA extraction. N.B.The test concentration of the mine tailing sample was 33,3 %. Soil total and acetate extractable concentrations were not determined from the diluted sample. Acetate extractable As concentration of R5.5 was below detection limit (3 mg/kg).

9. DISCUSSION AND CONCLUSIONS

Contaminated soils normally contain several contaminants simultaneously, and samples with pure arsenic contamination are rather uncommon. Samples for this project taken from the wood preservation area also contained Cu and Cr as expected, while samples from mine tailing contained very high concentrations of As and some Pb and Zn. Samples representing naturally As-rich areas contained As from 3 to 111 mg/kg (Table 9). When the fraction of As leached by Ammonium acetate-EDTA was compared to the total concentrations, there was a significant difference in the leaching behavior between the samples types. Approximately 30 % was leached from the mine tailing samples, while less than 10 % leached from the CCA-soils and uncontaminated soils.

In general, toxicity was shown by some test methods in all three sample types (Table 8). The sensitivity of the methods varied markedly. It is well known that species respond with different sensitivity to different harmful compounds. In this study, rye grass germination was the least sensitive test, since the effects detected were less than 20 %. On the other hand, lettuce germination was inhibited by other samples, except those from the mine tailing. According to duckweed growth inhibition tests and the RET -assay, there seemed to be harmful compounds that were easily leached into the growth medium or water. Plant effects are essential in assessing the ecotoxicity of soil samples. Germination tests are easy to perform, although minor responses may be expected, especially for metal contaminates soils. Duckweed growth inhibition proved to be a suitable method, demonstrating visible and clear effects. By this method, the toxicity of the contaminated soils was also clearly more severe than the mild effects caused by the naturals soils. The plant toxicity methods used were relatively simple to perform, and if possible, plant growth and life cycle tests should also be considered when planning large scale evaluation of risks.

Using the results of chemical analyses, a representative set of samples was chosen for soil invertebrate tests. Soil animals did survive in most of the samples, while reproduction was already affected by diluted samples. Earthworms were more sensitive than enchytraeids. For earthworms, the mine tailing samples without dilution were not a suitable living medium. Therefore, only diluted samples gave reliable results. The soil material was powder-like, sticking to the skin and obviously impeding the normal movement of the worms. Since the concentrations-response curve was very steep, both in the acute and in the reproduction phase of the tests, the EC-values could not be calculated for all samples. A preliminary test is recommended by the ISO standard, and it would possibly have assisted in choosing a more narrow range for the acute test, but still, the reproduction phase would have been out of range. These methods are quite time-consuming and therefore double testing is not an option in practice.

The highest concentrations of arsenic in earthworm tissue without acute toxic effects were approximately 350 mg/kg for both spiked soil and field soils (Fig. 12,Table 10). This is about 300 times the background concentrations of the worms taken directly from the basic culture (Table 9). Accumulation behavior of As from spiked soil to worm tissue differed from the field soils. An increase of As in tissue was almost linear up to soil arsenics value of 20 mg/kg in the spiked artificial soil and reaching a tissue arsenic value of more than 200 mg/kg. However, in natural As-rich soil (sample M5) containing 111 mg As/kg, a tissue concentration of only 30 mg/kg were determined (Fig. 12 and 13). Accumulation from the CCA-soils to worm tissue followed the pattern of the spiked soil (data not shown). In the control, the concentrations of As, Cd, Co and Cr in earthworms living on OECD artificial soil decreased with depuration time, while Cu an Zn concentrations remained constant, indicating the capability of tissue concentrations regulation of these essential metals. Although the purpose of this study was not to investigate bioconcentration of

arsenic, arsenic did not seem to accumulate in the earthworm tissue during the 4 weeks test period. This conclusion was based on the fact that the ratio of As in earthworm tissue/soil was less than 1, when measured from the animals depurated for 24 hours (Tables 7 and 10).

Our results of arsenic concentrations in earthworm tissue were determined only at one time point namely, at the end of the acute phase of the test. The possible bioconcentration of arsenic as a function of time could not be determined. Assuming that there are no fundamental differences in accumulation over time between earthworm species, the 28 d exposure should be long enough to reach the maximum non-lethal tissue concentration (Meharg et al., 1998). Actual bioconcentration could not be observed in our study. This is in contrast to the results of Fisher and Koszorus (1992) from *Eisenia fetida* studies but in accordance with the results that used other species (see Table 5).

Toxicity results from several methods should be interpreted together, all available physical and chemical data should be collected and sophisticated statistical methods should be used for detecting the cause and effect relationships considering multiple contaminants. If there were no time and resource limitations, one would test a large number of samples using a test battery of several tests to meet the requirements of statistical analyses. Ecotoxicity tests measure the effect of all harmful compounds and unfavorable physical properties of the ambient medium. Responses of test species to contaminants can vary. Therefore, simple correlation between the concentration of one element and toxicity are not very informative. Seed germination and Lemna growth inhibition results of the CCA soils and mine tailings were used to determine the cause and effect relationship of arsenic and toxicity. Results from the natural soils were too few and variable to be used in statistical analyses. It turned out that arsenic was the major contributor of the effects on lettuce seed germination and that toxicity could not be explained by acetate soluble arsenic. The same conclusion can also be made from the earthworm tissue concentrations of As compared to those in test soils after acetate leaching and total concentrations in soil (Fig. 13). This emphasizes the complex nature of the bioavailability and that that the assessment of bioavailability should be based on both biological and chemical methods.

Summarizing the results of all the ecotoxicity results of the project soil, the CCA soils seemed to be the most toxic, as toxicity was shown both by direct contact and by water-mediated methods. According to seed germination tests, the key toxicant both in CCA-soils and mine tailing was arsenic. Natural soils showed variable toxicity, but the number of samples with elevated arsenic (3 soils) allowed for several explanations as the cause of toxicity. Based on the earthworm tissue concentrations, high arsenic concentrations would be found in soft-bodied soil invertebrates in areas with high soil arsenic content. In addition to arsenic toxicity, physical properties of the mine tailing material may prevent the thriving of soil organisms in the area. Pot worm reproduction in artificial soil was not always high enough and substitution of it with field control soil with similar properties as the test soils would enhance the interpretation of the results. Background information of natural soils as the culture medium for soil invertebrates would be of great value.

Careful planning of the testing strategy is important, as the selection of the methods depends, among other things, on the purpose of the study such as risk assessment, remediation, monitoring, mapping of the contaminated area etc. The need for testing should also be assessed by first reviewing the existing chemical, biological and ecological data, time scale of the work, availability of methods and facilities and other resources. Interpretation of toxicity test results may be seemingly difficult, but keeping in mind the development of risk based limit values, the direct measurement of harmful effects would give a more relevant view of the effects of all harmful compounds than comparison of chemical concentrations to those assessed as hazardous for individual compounds.

10. SUMMARY

This report belongs to the series of publications of RAMAS –project, and is a part of Task 3 "Risk analyses". The goals of the project are to assess the environmental risk induced by the natural and anthropogenic arsenic, and to give recommendations for risk management procedures for the Pirkanmaa region. This report describes ecotoxicity of arsenic in soils based on the review of recent literature and on the ecotoxicological test results obtained during the project for samples taken from RAMAS field sites. The main purpose of the experimental work has been to assist the ecological risk assessment. This report has been prepared in SYKE during 2005-2007.

Data on occurrence, cycling, chemical properties, and concentrations of arsenic were collected to assist the experimental work. Interactions of arsenic, soil and organisms living in and on the soil is influenced by complicated processes comprising both biotic and abiotic factors such as soil structure, particle size, cation exchange capacity, pH, temperature, organic matter, concentration of phosphate and other compounds.

Biological methods can be used to measure the environmental hazard of chemicals and field samples. Bioassays with earthworms and plants represent direct contact tests or solid-phase tests. Standardized tests have been developed for better comparison of test results. There are standard methods for survival and reproduction of soil animals, and for germination and growth of plants. Earthworms are one of the key species in soil cultivation and responsible for the mixing of soil constituents and maintenance the fertility and structure of soils and recycle nutrients. Plants are primary produces supporting other forms of life. Hence, these species are well suited as surrogates to monitor and assess soil quality.

In the second part of this report, the objective was to examine arsenic contaminated soil sites with both chemical and ecotoxicological methods. Nineteen soil samples were collected from an old wood preservative area, a mining tailing and from areas where high background concentrations of arsenic have been observed. Samples were pretreated by two extraction methods before chemical analyses. Aqua regia extraction was used for measurement of the total arsenic and metal concentrations, and ammonium acetate-EDTA extraction for the bioavailable fraction of the elements. Total arsenic concentrations varied from 3 mg/kg in the natural soils to more than 4000 mg/kg in contaminated soils. Ammonium acetate-EDTA solution extracted ca. 7 % of arsenic from CCA –soils and 25 % from the mine tailing samples, while from natural soils the extracted concentrations were under the detection limit (3 mg/kg) in all but two samples.

For toxicity testing, different solid phase tests were used; two species for germination tests (ryegrass *Lolium multiflorum* and lettuce (L*actuca sativa*) and two survival and reproduction tests of soil invertebrates (earthworms *Eisenia fetida* and pot worm *Enchytraeus albidus*). Germination tests showed that rye grass germination was affected only slightly, and inhibition was less than 14 %. Effects on lettuce germination were more evident, with inhibition up to 70 % being recorded.

According to the earthworm and pot worm survival and reproduction tests, earthworms were more sensitive than pot worms, showing higher mortality and lower reproduction compared to the latter species. Samples did not affect survival of the enchytraeids, but reproduction was decreased in the presence of the natural soils, CCA-soil and mine tailing samples. Based on the earthworm test results, the difference between natural soils and CCA-soils was more evident. CCA-soils inhibited reproduction significantly. Only one mine tailing sample diluted with artificial soil was studied, because this material turn out to be unsuitable as a cultivation medium of earthworms. In this 50 %

dilution, the effects on earthworm survival and reproduction were of the same order as for CCA-soils. Calculation and interpretation of the results was complicated by the very steep concentration – effect curves. Reproduction could be changed from 100% to 0% from one dilution to the next, although the dilution ratio was only 1.3. Material from the earthworm tests was used to analyze the arsenic concentrations of the tissue. After 4 weeks' exposure, the maximum tissue concentrations were nearly 400 mg As/kg, while the background in the control animals was ca 1 mg/kg.

Terrestrial tests measure direct biological effects of soil on the test organism. Since leaching to water and mobility of harmful compounds are of environmental importance, aquatic bioassays are also useful in assessing water-mediated effects. Two aquatic tests, duckweed (*Lemna minor*) growth inhibition and an enzymatic *in vitro* test RET (reverse electron transport) test were used in this project. According to these methods, duckweed growth was clearly inhibited in the presence of CCA-soils and mine tailing samples, but the effects on natural soils were insignificant. Nearly all water extracts of the samples inhibited RET to some extent, but again the CCA-soil was the most toxic.

Based on the results, it can be summarized that biological methods are justified in assessing the harmful effect and bioavailability. Distinguishing the effect of arsenic from those of other elements and compounds was not easy, notably because of the limited number of the samples that could be tested during the project. Nevertheless, considering seed germination, the CCA-soils and mine tailing material, it could be concluded that arsenic was the main cause of ecotoxic effects.

11. YHTEENVETO

Tämä raportti kuuluu RAMAS-projektiin ja on osa hankkeen Task 3 "Riskianalyysit" -osiota. Hankkeen tavoitteena on arvioida luontaisen ja ihmisen toiminnan aiheuttaman arseenin terveys- ja ympäristöriskejä Pirkanmaan alueella. Se antaa myös suosituksia riskin hallinta-menettelyihin. Tämä raportti kuvaa arseenin ekotoksisuutta maaympäristössä kirjallisuuskatsauksen muodossa ja RAMAS-projektin tutkimuskohteista otettujen näytteiden tutkimustulosten valossa. Kokeellisen tutkimuksen päätavoite on ollut tuottaa uutta mittaustietoa ekologisen riskinarvioinnin käytettäväksi. Raporttia on tehty vuosina 2005-2007 SYKEssä.

Kirjallisuudesta kerättiin taustatietoa kokeellista osaa varten maaympäristön arseenin esiintymisestä, kierrosta, kemiallisesta rakenteesta, pitoisuuksista jne. Vuorovaikutukseen arseenin, maaperän ja eliöstön välillä vaikuttavat monet tekijät, kuten arseenin kemiallinen muoto ja alkuperä, arseenin muuntuminen. Vuorovaikutukseen vaikuttaa myös maaperän rakenne, maatyyppi, raekoko, kationinvaihtokapasiteetti, pH, lämpötila, orgaanisen aineksen määrä, fosfaatin ja muiden yhdisteiden pitoisuus.

Kirjallisessa osassa kerättiin tietoa myös arseenin ympäristövaarallisuuden mittaamiseen soveltuvista biotesteistä. Erityisen sopivina arseenin myrkyllisyyden mittaamiseen pidetään ns. kiinteäfaasi- eli suorakontaktitestejä. Kiinteäfaasitestejä ovat maaperäeläinten kuolevuutta ja lisääntymistä mittaavat testit sekä kasvitestit. Menetelmistä on laadittu kansainvälisiä standardeja, joilla pyritään saamaan vertailukelpoisia tuloksia. Lieroilla on olennainen rooli maaperän rakenteen muokkaajana ja ravinteiden kiertokulussa. Kasveilla on tärkeä tehtävä kaiken muun elämän ylläpitäjänä, jolloin ne lierojen ohella sopivat hyvin biologiseksi indikaattoriksi.

Kokeellisessa osassa tutkittiin kemiallisin ja ekotoksikologisin menetelmin yhdeksäntoista maanäytettä, jotka olivat vanhalta puunkäsittelyalueelta, kaivosalueelta sekä alueilta, joilla oli todettu luontaisesti korkeita arseenipitoisuuksia. Maanäytteiden kemiallista pitoisuusmääritystä varten käytettiin esikäsittelynä kahta erilaista uuttoa. Kokonaispitoisuuden määrittämiseksi tehtiin näytteille kuningasvesikäsittely. Maanäytteiden kokonaisarseenipitoisuudet vaihtelivat luonnon maanäytteiden matalista pitoisuuksista (3 mg/kg) pilaantuneen maan korkeisiin pitoisuuksiin (>4000 mg/kg). Biosaatavan arseenin määrittämiseksi käytettiin ammoniumasetaatti-EDTA-uuttoa. Ammoniumasetaatti-EDTA -uutolla saatu fraktio oli 7 % CCA-maiden sisältämästä arseenista ja 25 % kaivoksen rikastehiekan sisältämästä arseenista. Asetaatti-EDTA-liuos uutti luonnon maista arseenia hyvin vähän (alle kemiallisen määritysrajan, 3 mg/kg).

Maanäytteiden ympäristömyrkyllisyyttä testattiin sekä suoraan maanäytteistä tai niiden laimennoksista että uutteista vesieliöiden käyttöön perustuvilla biotesteillä. Kiinteäfaasikokeissa käytettiin kasvien itävyystesteissä kahta kasvilajia: raiheinää (*Lolium multiflorum)* ja salaattia (*Lactuca sativa*). Lisäksi tutkittiin eräillä näytteillä vaikutuksia kahden maaperän selkärangattoman, lieron (*Eisenia fetida* ja änkyrimadon *(Enchytraeus albidus)*, kuolevuuteen ja lisääntymiseen. Itävyystestien tulosten perusteella raiheinän itäminen aleni hieman (inhibitio <14 %), mutta salaatin itäminen väheni selvästi (suurimmillaan 70 %:n inhibitio).

Maaperäeläinten kuolevuus- ja lisääntymistestien tulosten perusteella lierot olivat änkyrimatoja herkempiä. Änkyrimatojen kuolevuuteen ei näytteillä ollut vaikutuksia, mutta lisääntyminen väheni sekä luontaisten näytteiden, CCA-maiden että kaivosjätteen läsnä ollessa. Lierojen lisääntymistestissä erot luontaisten maiden ja CCA-maiden välillä olivat selvemmät, ja CCA-maat osoittautuivat haitallisiksi ja luonnonmailla havaittiin lieviä vaikutuksia. Kaivoksen rikastehiekka ei soveltunut laimentamattomana lierojen elinalustaksi, joten näistä tutkittiin vain yksi näyte laimennettuna keinomaalla (50 % laimennos). Tällöin vaikutukset olivat samaa luokkaa kuin CCA-mailla. Tulosten tulkintaa vaikeutti se, että vaikutukset olivat jyrkästi riippuvaisia pitoisuudesta. Kahden laimennoksen välillä lisääntyminen saattoi estyä 0 -100 %, vaikka näytteiden välinen laimennussuhde oli 1.3. Lierotestien materiaalia käytettiin myös eläinten kudoksiin kertyvän arseenin määrityksiin. Aikuisten eläinten kudoksiin kerääntyi neljän viikon altistuksen aikana arseenia enimmillään lähes 400 mg/kg CCA-maissa eläneissä lieroissa, kun taustapitoisuudet kontrollieläimissä olivat noin1 mg/kg.

Kiinteäfaasi testit eli terrestriset testit kuvaavat arseenin suoria biologisia vaikutuksia maaperän ja testieliön välillä. Toinen tekijä arseenilla pilaantuneiden maiden riskinarvioinnissa on arseenin liukeneminen veteen ja arseeniyhdisteiden kulkeutuminen sen mukana. Vesieliöiden käyttöön perustuvilla testeillä voidaan arvioida veden välityksellä tapahtuvien haittavaikutusten määrää. Tässä työssä käytettiin kahta vesiympäristön biotestiä, kelluvan pikkulimaskan (*Lemna minor*) kasvun estymistestiä ja entsymaattista (reverse electron transport, RET) *in vitro* testiä. CCA-maa ja kaivosjäte estivät selvästi pikkulimaskan kasvua, kun taas luonnon maiden vaikutus oli vähäinen. Lähes kaikki näytteet inhiboivat RET-testissä jonkun verran ja CCA-maat olivat erityisen myrkyllisiä.

Tuloksista voitiin todeta, että haitallisuuden ja biosaatavuuden tutkimiseksi biologiset menetelmät ovat hyödyllisiä. Arseenin vaikutusten erottaminen muiden haitta-aineiden vaikutuksista on hankalaa, kun tutkittavat näytemäärät eivät käytännön syistä yleensä voi olla kovin suuria. CCA-maita ja kaivoksen rikastehiekkaa tutkimalla saatiin kuitenkin todisteita siitä, että arseeni on suurimpana tekijänä ainakin salaatin itävyyden estymiselle.

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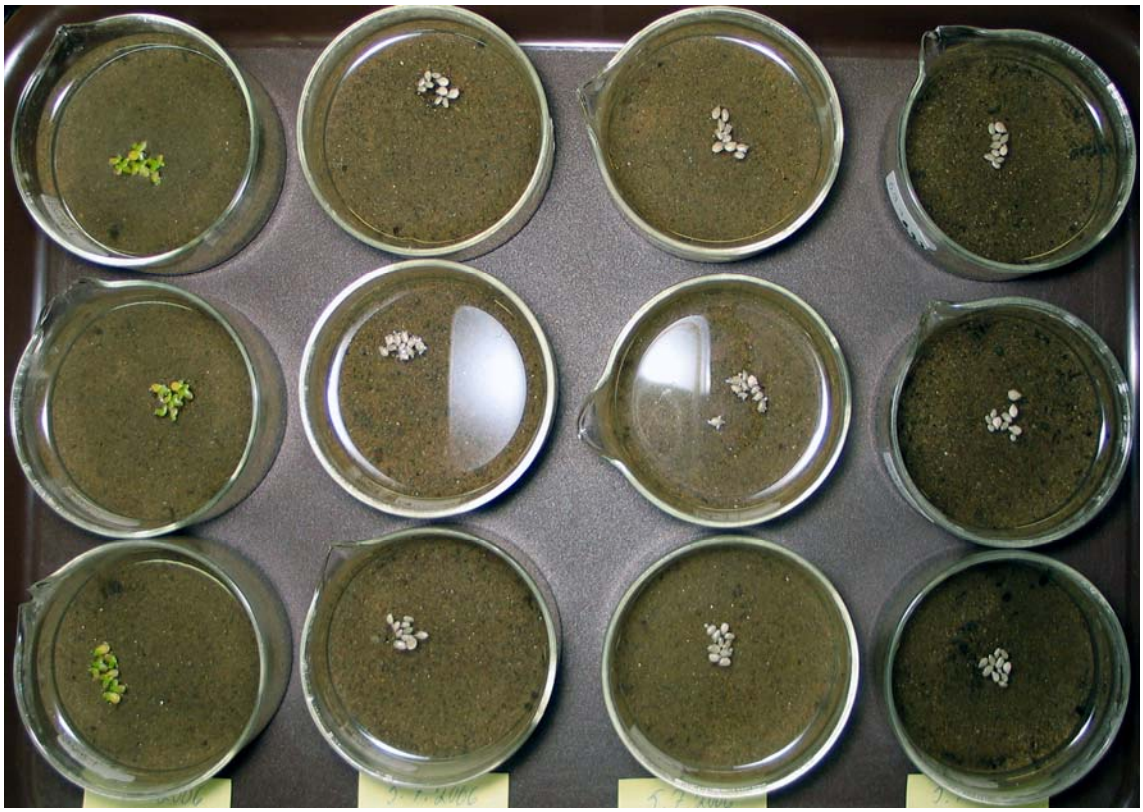
APPENDIX.
Photographs taken at the end of the duckweed test

Duckweeds *Lemna minor* were grown in nutrition solution in the presence of RAMAS soil samples for 7 d. Fifty grams of the solid samples were put on the bottom of test vessels and 100 ml of standard nutrition solution (ISO/FDIS 20079) was added into the vessels. All necessary ingredients are present in the medium to allow normal growth of the plants. Control vessels contained natural sand instead of test soil. Effects such as decreased number of fronds and decreased amount of chlorophyll, chlorosis and necrosis are clearly visible.

a) Natural soil M1 in triplicate assays (left) and six control vessels (right)

APPENDIX, continued

b) CCA-soil samples R1.3, R2.1,R3.2 and R5.3 in triplicate assays from left to right



APPENDIX, continued

c) Six control samples (left) and mine tailing sample L1 and L2 in triplicate assays

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The project will produce a number of Technical Reports. The following reports have been published:

1. Natural Occurrence of Arsenic in the Pirkanmaa Region in Finland
2. Anthropogenic Arsenic Sources in the Pirkanmaa Region in Finland
3. Arseenista aiheutuvien riskien hallinta Pirkanmaalla – Esiselvitys ohjaus keinoista ja teknisistä menetelmistä riskien vähentämiseksi (Management of arsenic risks in the Pirkanmaa region – Survey of available risk management instruments and tools)
4. Arsenic and other elements in agro-ecosystems in Finland and particularly in the Pirkanmaa region
5. A transport model of arsenic for surface waters - an application in Finland
6. Arsenic Ecotoxicity in Soils

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