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Tuomas Lukkari

Earthworm Responses to Metal Contamination

Tools for Soil Quality Assessment

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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

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ABSTRACT

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Responses of earthworms to soil metal contamination were studied in field and laboratory experiments. In the field, earthworm communities and metal bioavailability to earthworms along contamination gradients were surveyed in three areas located close to metal industry in Finland. In the laboratory, toxicity tests and experiments using field soil simultaneously contaminated with Cu and Zn, and with different earthworm species and populations with different exposure histories were established to determine species- and population-specificity in responses to metals. In two of the three field areas studied, soil metal concentrations close to the emission source were high enough to induce clear harmful effects on earthworm total number, biomass, and diversity. In the laboratory experiments, earthworms avoided contaminated soils with metal concentrations lower than those needed to induce biochemical responses. Increasing soil metal concentration reduced earthworm activity, interfering with reproduction and decreasing biomass, and finally caused mortality. *Aporrectodea tuberculata*, the species used in most of the experiments, tolerated higher metal concentrations in the field soil than in the standardised artificial soil. A species used in standardised tests, *Eisenia fetida*, appeared to be more tolerant of metals and to regulate metal body burdens more strictly than *A. tuberculata*. Ecologically different species avoided metal contaminated soils differently, partly clarifying species-specific differences found in the field. Earthworms of a population with long-term exposure to metal contamination seemed to tolerate higher metal concentrations than a population without earlier exposure. In addition, earthworms seemed to reduce the mobility and bioavailability of metals in the soil. When interpreted with care, earthworm data shows the effects of metal contamination and other soil conditions. Results of this study emphasise that it is important to consider ecological characteristics and possible tolerance of the species, and even those of the population, used in ecotoxicological tests and field surveys. In summary, the inclusion of earthworm responses to support soil chemical analyses is highly recommended when potential harmfulness of anthropogenic substances and soils are estimated in risk assessment procedures.

Key words: Cu and Zn; earthworms; ecotoxicity tests; metal contamination; quality assessment.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. I have personally taken part in the original planning of the experiments and I have performed the majority of the work described in each article. I have also written the first drafts of all articles, which were then completed in co-operation with the other authors.

I Lukkari, T., Taavitsainen, M., Väisänen, A., & Haimi, J. 2004. Effects of heavy metals on earthworms along contamination gradients in organic rich soils. Ecotoxicology and Environmental Safety 59: 340-348.

II Lukkari, T., Taavitsainen, M., Soimasuo, M., Oikari, A., & Haimi, J. 2004. Biomarker responses of the earthworm *Aporrectodea tuberculata* to copper and zinc exposure: differences between populations with and without earlier metal exposure. Environmental Pollution 129: 377-386.

III Lukkari, T., Aatsinki, M., Väisänen, A. & Haimi, J. 2004. Toxicity of heavy metals assessed with three different earthworm tests. Manuscript, submitted to Applied Soil Ecology.

IV Lukkari, T. & Haimi, J. 2004. Avoidance of Cu and Zn contaminated soil by three ecologically different earthworm species. Accepted, Ecotoxicology and Environmental Safety.

V Lukkari, T., Teno, S., Väisänen, A. & Haimi, J. 2004. Responses of two earthworm populations with different exposure histories to metals – consequences in decomposition processes and metal availability. Manuscript, submitted to Soil Biology and Biochemistry.

1 INTRODUCTION

"Take us worms, for example. We till, aerate, and enrich the earth's soil, making it suitable for plants. No worms, no plants; and no plants, no so-called higher animals running around with their oh-so-precious backbones!" father worm say to the little worm.
-Gary Larson (1998): There's a hair in my dirt – A worm's story-

1.1 Background for the thesis

This thesis deals with the important, complicated, and current issue of the impacts of harmful contaminants on the environment. Soils, which are composed of minerals, organic substances, water, air, and living organisms, are the sites of many important processes, such as decomposition of organic matter and nutrient mineralisations which occur mainly through the function of soil organisms and form the basis for terrestrial life. A major part of harmful anthropogenic substances finds its way into the soil organic layer, making it a sink for contaminants. These contaminants, in turn, may negatively affect soil organisms, and thus be reflected in all soil processes. Hereby, the responses of soil organisms, such as decomposer animals, to soil contamination can give relevant information on soil and ecosystem health. In this thesis, responses of important decomposers, earthworms, to soil metal contamination were studied, and how their responses can be used as a tool for assessing harmfulness and possible risks of contaminated soils, was evaluated. Because this issue requires contributions from many fields of science, this thesis is multidisciplinary, utilizing approaches and methods from ecology, ecotoxicology, and chemistry.

University of Jyväskylä has a long tradition of studying soil organisms and their ecology. Since the late 1970´s, investigations have been made to determine the effects of human influences, such as silviculture and fertilisation on soil decomposer communities and to clarify the role of soil fauna in decomposition processes and nutrient cycles (e.g. Huhta 1984). The main theme in the studies of the "Soil Group" has been to find out the functional importance of soil organisms in ecosystem processes (Setälä 1990, Haimi 1993, Mikola 1997, Laakso 1998, Liiri 2001, Sulkava 2001, Nieminen 2002, Rantalainen 2004, Räty 2004). In addition, responses of soil biota to anthropogenic disturbances, such as harmful organic chemicals (Salminen 1996), pesticides (Martikainen 1998), and forest management practices (Siira-Pietikäinen 2002), have been studied. This thesis constitutes a consistent continuum to the previous studies, concentrating on one group of harmful contaminant, metals.

1.2 Metals in soils

1.2.1 Metal contamination

Metals from anthropogenic sources are widely spread in the atmosphere and in aquatic and terrestrial environments. They form a major group of compounds involved in soil contamination and cause damage to ecosystems close to emission sources by affecting the abundance, diversity, and distribution of organisms (Lobersli & Steinnes 1988, Kozlov et al. 1993, Haimi & Siira-Pietikäinen 1996, Yaron et al. 1996, Derome & Nieminen 1998). Potential metal contaminants are produced in mining, metal, pigment, and chemical manufacturing, as well as in combustion of fossil fuels, including the exhaust emissions of motor vehicles (Tarradellas et al. 1997). Metals, such as arsenic, cadmium, chromium, copper, mercury, and zinc, play a significant role in soil contamination and they are often present simultaneously in the same area. Regarding their toxicity, it is well known that several metals, especially arsenic, cadmium, chromium, and mercury, pose a potential risk to the environment even in low concentrations (Yaron et al. 1996). On the other hand, metals like copper and zinc are essential trace elements for organisms, becoming toxic above certain concentrations and exposure times (Hopkin 1989). In addition to chemical properties and concentration, toxicity and other negative effects of metals are related to soil properties and other environmental conditions (Hopkin 1989, Allen 2002, Lanno et al. 2004).

Most of the emitted metals finally end up in surface soil layers. They bind especially to organic matter, which is abundant in northern forest soils (Allen 2002). Binding reduces metal mobility and bioavailability but, on the other hand, metals are bound in those parts of ecosystems where essential biological processes, such as decomposition and nutrient mineralisation, take place. In contrast to harmful organic compounds, metals do not ultimately degrade and disappear from soils even if their release to the environment can be restricted (Brusseau 1997). Therefore, metal contamination really has long-term effects on soil organisms and processes: in the vicinity of many emission sources, soil organisms are exposed to metals over many generations.

1.2.2 Availability of metals to soil organisms

Traditionally, quality of metal contaminated soils is based on total soil metal concentrations (Allen 2002). This measure usually provides little information on the bioavailability and mobility of metals in soil and thus ignores the actual exposure of soil organisms to metals. Bioavailabity can be defined as "a measure of the potential of an element or substance for entry into biological receptors" (Lanno et al. 2004). It is also specific to the receptor (organism), the route of entry, time of exposure, and the matrix containing the contaminant. In addition, measurements of bioavailability may be either direct or indirect and, on the other hand, chemical or biological (Lanno et al. 2004). It should be noted that soils form a very heterogeneous environment having varying spatial and temporal gradients of physical and chemical characteristics (e.g. particle size, water and organic matter content, pH). These characteristics together with environmental conditions, and physiology and behaviour of the organisms, determine the bioavailability of metals or other substances in soils to the organisms. In addition, metal source and equilibration times in soils affect their bioavailability (Peijnenburg et al. 1997). It has been shown that metal-spiked soil in laboratory experiments is more toxic to soil organisms than the equivalent metal concentrations in field soils (Streit 1984, Langdon et al. 2001b).

Several extraction methods have been proposed to predict metal bioavailability but none of them have been accepted as its absolute measure and hence, a reliable basis for assessing harmfulness of metal contaminated soil (Peijnenburg 2002). Commonly used analytical methods were developed and recommended for the analysis of sediments and agricultural soils (ISO 11466 1995, Karstensen 1996). Extraction of metals from soil samples is usually time-consuming and quite expensive, requiring special laboratory instruments and digestion with acids such as hydrofluoric and perchloric acid (ISO 11466 1995). Metals, like copper and zinc, may be present in soils in several chemical forms, each having a specific mobility and availability to soil organisms (Brusseau 1997). Instead of a single extraction, speciation of metals in the soils with a sequential extraction procedure offers more extensive knowledge of the proportions of differently behaving forms of metals (Tack & Verloo 1996, Ure 1996). Sequential extraction using increasing strength of extraction solution fractionates metals into forms of different solubility and mobility, and may therefore act as an improved tool for predicting metal availability to soil biota, movement in the soil profile, and transformation between different forms in soils (Cooksey & Barnett 1979, Tessier et al. 1979, Luo et al. 2003).

While the environmental fate of metals and their biological impacts in changing environmental conditions are difficult to predict, risk assessment for soil organisms and contaminated soils should not be based on total metal concentrations only. Therefore, it has been proposed that available metal concentration analysed e.g. with sequential extraction procedure should be brought into common use in ecotoxicological studies and laboratory practice (Ure et al. 1993, Van Gestel & Weeks 2004).

1.2.3 The problematic definition of "heavy metals"

The term "heavy metals" is popularly used in the environmental sciences, in many publications and legislation dealing with harmfulness and safe use of different substances which consist of metal components. It is typically used as a common denominator for metals and semimetals (metalloids), associated with contamination and potential ecotoxicity. There is a general confusion regarding the use and meaning of the term, which can alter from one set of regulations to the other and can be used without specifying particular metals. In fact, it is not proper to assume that all "heavy metals" have highly toxic or ecotoxic properties since they do not have an axiomatic value of toxicity or harmfulness. Based on the arguments raised by Duffus (2002) and Hodson (2004), the term "heavy metals" in this thesis has been altered to simply "metals" or direct specification of the used metal.

1.3 Tests for harmful effects of metals on terrestrial decomposer organisms

Currently, harmfulness of metals and other anthropogenic substances to decomposer organisms are assessed with various methods from chemical analyses to different kinds of biotests and field monitoring, mainly measuring mortality or reproduction of selected species or estimating number and diversity of organisms in the contaminated field site (Karstensen 1996, Belotti 1998, Løkke & Van Gestel 1998). Some of these tests have been standardised and some are undergoing standardisation processes in the Organisation for Economic Cooperation and Development (OECD), the International Organisation for Standardisation (ISO), and the European Committee for Standardisation (CEN). Standard laboratory tests have also been applied for assessing the toxicity of contaminated soils. Standardised acute toxicity (ISO 11268-1 1993) and reproduction (ISO 11268-2 1997) tests on earthworms have proven to be useful tools in assessing the toxicity and possible risks of soil contaminants (Løkke & Van Gestel 1998). In addition, other methods measure functioning of the soil microbial communities by testing soil respiration and certain enzyme activities. These tests are certainly time-saving and valuable tools when assessing the toxicity of specific elements or substances or contaminated soils to decomposers. However, some important sublethal responses may remain overlooked; e.g. the tests do not provide information on how individuals may react when facing contaminants in their environment. Sublethal responses, often called "early warning signals", may in fact, give very important information when assessing the environmental risks of contamination (Van Straalen & Denneman 1989). Especially in field monitoring, the results from standardised tests often are also difficult to interpret because of the myriad of environmental abiotic and biotic factors that are always contributing to the parameters under focus and are insensitive to low level contamination leading to under protection and management (Løkke & Van Gestel 1998). Hence, the standardised tests may not offer enough information on soil contamination to predict effects in field populations and ecosystem level processes (Cairns 1984, Løkke & Van Gestel 1998).

Microcosms have been developed to decrease the gap between simple laboratory studies and data derived from field conditions (Sheppard 1997). Microcosms are replicable, small (either intact or somewhat treated) samples of diverse soil subsystems enclosed in experimental units. They help in revealing the responses of organisms to harmful compounds in their natural environment and also in tracing how these responses are reflected in processes at higher levels of biological organisation, such as regulation of community structure, decomposition processes, and primary production (Moore et al. 1996, Verhoef 1996). Because microcosm studies measure many parameters and endpoints at the same time in the same environment, they are intermediate to laboratory and field studies, offering more information for higher tiers of risk assessment than standardised single species toxicity tests (Sheppard 1997, Olesen & Weeks 1998, Römbke et al. 2004, Weyers et al. 2004).

Organisms may respond to environmental contamination either by acclimatisation or through genetic adaptation over several generations (Bengtsson et al. 1992, Reinecke et al. 1999). Survival of populations in contaminated environments requires increased resistance of exposed individuals or other mechanisms minimising their exposure (Spurgeon & Hopkin 2000, Langdon et al. 2001a). Resistance to harmful contaminants may result from increase in detoxification or excretion processes or decrease in cuticular or intestinal uptake (Dallinger 1993, Morgan et al. 1993). On the other hand, changes in behavioural or life-history traits in patchily contaminated environments can diminish the actual exposure and thus, the effects of contaminants on soil animals (Tomlin 1992, Stephenson et al. 1998, Morgan & Morgan 1999). For example, it should be also emphasised that many organisms can distinguish contaminants in the soil, and avoid them and any possible harmful physiological or toxic effects (Peijnenburg 2002). Importantly, it has been proposed that directional selection for resistance will result in a concomitant increase in fitness costs. Benefits of resistance may be reduced by negative impacts on other fitness components, such as decreased allocation of resources to reproduction or growth (Eckwert & Köhler 1997). These potential costs may also be reflected in life-history traits and activity of organisms, which may affect the overall properties and efficacy of the decomposer community and have potential impacts for the whole contaminated habitat and ecosystem.

1.4 Rationale of studying soil contamination using biological tests

Traditional physical and chemical analyses, while being important part of assessing fate of the contaminants in the environment, cannot by themselves fully answer questions about the ecotoxicity of potentially harmful elements or substances. Chemical measurements report only elements or substances that are analytically determinated. Even if all chemical forms can be measured, understanding of toxicant interactions (i.e. additive, synergism, or antagonism) is unavailable for predicting biological responses (Khalil et al. 1996, LaPoint & Waller 2000). It is also important to consider that organisms, which are continually exposed to several contaminants and other stressors under field conditions, do not reflect only current conditions but also past conditions and their cumulative effects on the organisms.

The rationale of using biological tests and bioindicators for assessing potential harmfulness of contaminants or contaminated soils is that the organisms tested or monitored provide the best reflection of the actual fitness of their habitat and of changes therein (Didden 2003). They can also detect the potential for harmful effects on organisms before those effects occur in the field or on a large scale. Therefore, in principle, it is possible to detect habitat and environment change by looking at the organisms and defining the presence of unknown or unexpected stressors that could be otherwise overlooked (Didden 2003). Biological tools may provide an early warning of impending ecological change caused by contaminants by identifying biochemical, behavioural, and physiological responses of studied species and populations followed by contaminant exposure.

Biological tests and monitoring tools have many advantages which make them, together with chemical analyses, a useful and important part of assessing possible harmfulness and risks of soil contaminants. Soil organisms, as biological indicators, are exposed to background levels in their own habitat, and the concentration of the contaminants can usually be measured from the organisms. Thus, actual exposure and accumulation tendency can be determined, demonstrating whether the contaminants are bioavailable. Biotests can also evaluate the integrated harmful and toxic effects of all contaminants at the same time in the same medium. Hereby, biological tests and bioindicators together with chemical analyses give a natural, reliable, and repeatable view of the condition of the environment (Løkke & Van Gestel 1998, Markert et al. 2003).

1.5 Value of earthworms in soil ecotoxicology

In many soils, a major part of the energy and nutrients flow through only few or even one abundant decomposer animal species (Lee 1985). Responses of these key species are of crucial importance to the functioning of the ecosystem. Because earthworms are key species in many terrestrial ecosystems, their response to soil contamination is expected to be reflected in ecosystem functioning (Edwards & Bohlen 1996). Therefore, earthworms have been considered to be valuable animals for assessment of soil quality and contamination and useful tools in environmental monitoring: they are good indicators of the condition of soils (Van Gestel et al. 1997, Kula & Larink 1998, Paoletti 1999, Didden 2003). Earthworms have a great impact on decomposition activity, nutrient mineralisation, primary production, and soil structure maintenance, and they are also an important part of the terrestrial food chain (Lee 1985, Edwards & Bohlen 1996). They also reflect site-specific contamination levels because of limited mobility capacity and close relationships to different soil conditions, such as moisture content and pH (Edwards & Bohlen 1996). Earthworms are model organisms for ecotoxicology studies.

While burrowing, earthworms ingest large amounts of soil and are therefore exposed to contaminants through their intestine as well as through their skin. As a result, substances are concentrated from the soil into their body, making them ideally suited for assessing the bioavailability of many harmful substances (Morgan & Morgan 1999, Heikens et al. 2001, Lanno et al. 2004). It has also been observed that earthworms *Lumbricus rubellus* (Hoffmeister) and *Aporrectodea caliginosa* (Savigny) accumulate higher amounts of contaminants than other soil invertebrates such as isopods and millipedes (Pathirana et al. 1994, Hobbelen et al. 2004). Consequently, earthworms are expected to represent the worst case of toxicant availability. Moreover, earthworms are easy to handle and culture, and it is fairly practical to measure different repeatable parameters on earthworms, such as reproduction, growth, accumulation and excretion of substances, and biochemical responses. A further advantage is that earthworms can be used as representative organisms in both laboratory and field conditions.

1.5.1 From behavioural and physiological responses to ecosystem functioning

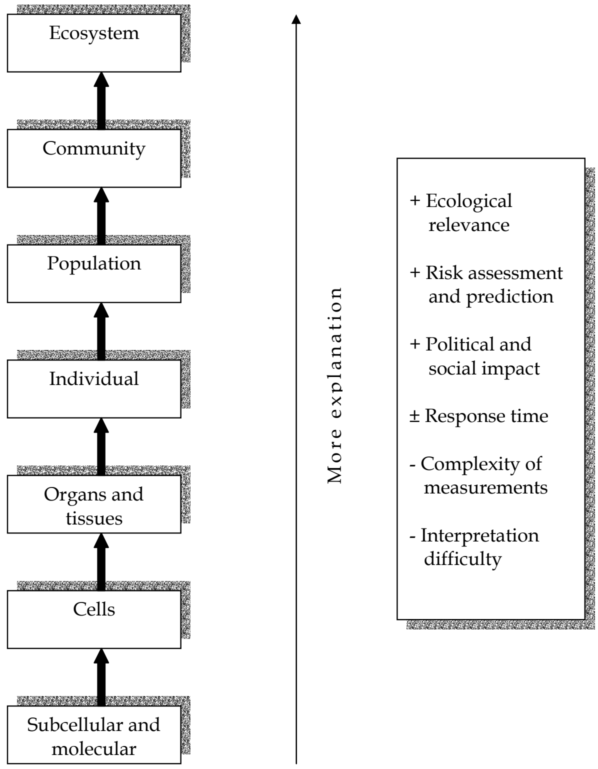

FIGURE 1
Biological organisation level at which bioindicators and –monitors can be identified, and they can be used as a tool to estimate the harmfulness of contaminants or contaminated soils to the organisms. General outlines, advantages (+) and disadvantages (-), are also shown. Adapted from Morgan et al. 1999.

Earthworm responses to soil contamination are identified at many levels of biological organisation, from molecular to ecosystem level (Spurgeon et al. 1994, Svendsen & Weeks 1997, Spurgeon et al. 2000, Reinecke et al. 2001, Morgan et al. 2002, Spurgeon et al. 2004; see Fig. 1). At the molecular and cellular levels the responses to stress factors include rapid and sensitive changes; in genetic material, in induction of stress proteins, in functioning of the nervous and immune systems, and in structure of cells and organelles (Didden 2003). These responses are commonly named biomarkers, which can be defined as "any biological response to an environmental chemical or substance at the individual level or below, demonstrating a departure from the normal status" (Timbrell 1998). In addition, if the molecular and cellular level response is expressed due to metal contamination, it would give important information, warning signals, for assessing the environmental harmfulness of the contamination (Huggett et al. 1992). At the individual and population levels, changes in accumulation, bioconcentration, abundance, biomass, reproduction, growth, behaviour, and morphology are expressed. Changes in community composition represent the community and ecosystem level responses (Morgan et al. 1999). However, it should be emphasised that decreases in e.g. survival and reproduction are not necessarily needed for changes in population or community dynamics and functioning of earthworms. Changes in life-history characteristics, resistance, and avoidance in earthworm populations from long-term contaminated areas may affect population or community dynamics.

Responses of earthworms to soil contamination may vary depending on the species and even the exposure history of the individuals (Ma 1988, Khalil et al. 1996, Reinecke et al. 2001). Possible acclimatisation of earthworms and selection for increased resistance during long-term exposure are the two important factors that can affect the results obtained from laboratory and field studies (Eckwert & Köhler 1997). Earthworm species inhabiting metal contaminated soils have species-specific behavioural characteristics, such as burrowing and feeding activities, which evidently affect their responses to metal contamination (Morgan & Morgan 1999, Reinecke et al. 1999). They are capable of decreasing possible toxic effects of metals by regulating and detoxifying their internal metal concentrations with varied proteins and enzymes and by avoiding the most contaminated soil patches by moving into less contaminated patches (Dallinger 1993, Mather & Christensen 1998, Langdon et al. 2001b, Schaefer 2003). Earthworms can perceive a diverse range of chemical stimuli to help them e.g. in the selection of food and in detecting adverse environmental conditions, such as changed soil acidity and harmful contamination. Acute chemical sensitivity of chemoreceptors and the ability for dispersal make the earthworms capable of avoiding unfavourable habitats, e.g. highly contaminated soil patches (Edwards & Bohlen 1996). In heterogeneously contaminated habitats, earthworms may avoid the most contaminated soil patches, restricting their impacts on soil processes in these areas, which can be reflected in the functioning of the whole ecosystem (Salminen & Sulkava 1996, Yeardley et al. 1996, Stephenson et al. 1998). Therefore, earthworm avoidance response may offer an ecologically relevant endpoint for ecotoxicological assessment of potentially harmful substances and contaminated soils.

1.5.2 Selection of tests species

Eisenia fetida (Savigny) and *E. andrei* (Bouché), standardised test species, while generally being neither less nor more sensitive to harmful compounds than other earthworm species (Kula & Larink 1998), may tolerate higher metal concentrations than other earthworm species, such as the widely distributed and abundant *Aporrectodea tuberculata* (Eisen) and *Lumbricus rubellus* in the northern hemisphere (Spurgeon et al. 2004). In addition, *Eisenia*-species live in habitats rich in organic matter, such as composts and dung heaps, not in normal soils as indigenous species (Edwards & Bohlen 1996). Thus, these species may not be ecologically most relevant species for all ecotoxicological studies, at least in northern conditions. More relevant test species might be e.g. *A. tuberculata*, *L. rubellus* and *Dendrobeana octaedra* (Savigny), which are common and abundant in many kinds of northern habitats and which have different ecological strategies (Terhivuo 1989, Edwards & Bohlen 1996). Therefore, in the ecotoxicological studies and risk assessment procedures, it is important to consider the species in addition to environmental conditions and properties of harmful substances.

1.6 Ecological risk assessment of contaminants in soils

Risk Assessment (RA) can be shortly described as assessment of the probability of a hazard being realised (Suter et al. 2000). Traditionally RA procedure includes four steps: hazard identification, exposure assessment, dose-response assessment and risk characterisation. The ultimate goal of RA is characterisation of adverse effects resulting from exposure to a hazard (Environment Agency 2003). A number of directive and regulatory regimes now require use of RA to assess industrial chemicals and substances through their life-cycle; from production to formulation and use, and ending with disposal (Commission of the European Communities 1996, Campbell et al. 1999, Candolfi et al. 2001, Commission of the European Communities 2003). Recently another supplementary approach, Ecological Risk Assessment (ERA), has been adopted for evaluating the likelihood of adverse effects on organisms, populations, and communities from a chemical present in the environment, since environmental issues have to be taken into better consideration in nearly all industrial frameworks (Fig. 2). ERA is changing rapidly with each new regime due to the introduction of new chemicals and substances to the environment as well as the introduction of new scientific information about possible harmful compounds. Thus, ERA has to be constantly developed in order to maintain its efficacy in environmental protection (Suter et al. 2000).

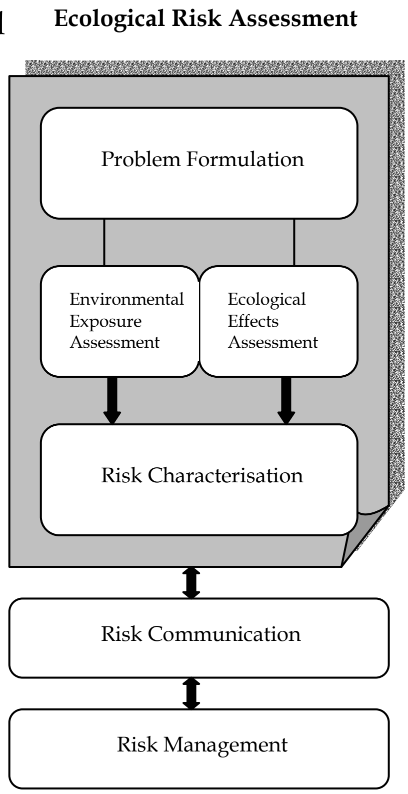

FIGURE 2
The basic framework for Ecological Risk Assessment (ERA), (Suter et al. 2000).

1.6.1 A tiered approach in Ecological Risk Assessment

A tiered approach, with clear procedures for conducting ERA, improves the usability and reliability of ERA for site-specific risk assessment by reducing uncertainties. It provides a practical match between available resources and the severity of the problem with rational and consistent decision making. The tiered approach allows for early identification of risks with lowered consumption of time, effort, and resources. The tiers help to identify those contaminated sites that represent potentially significant risks and to screen out sites where minimal risks are indicated. In general, the tiered approach uses physico-chemical, biological, and ecological information for assessing possible risks. First, physico-chemical properties of the soil regarding the contaminants present at the site, their forms, and concentrations are analysed. Then, biological data collected from the

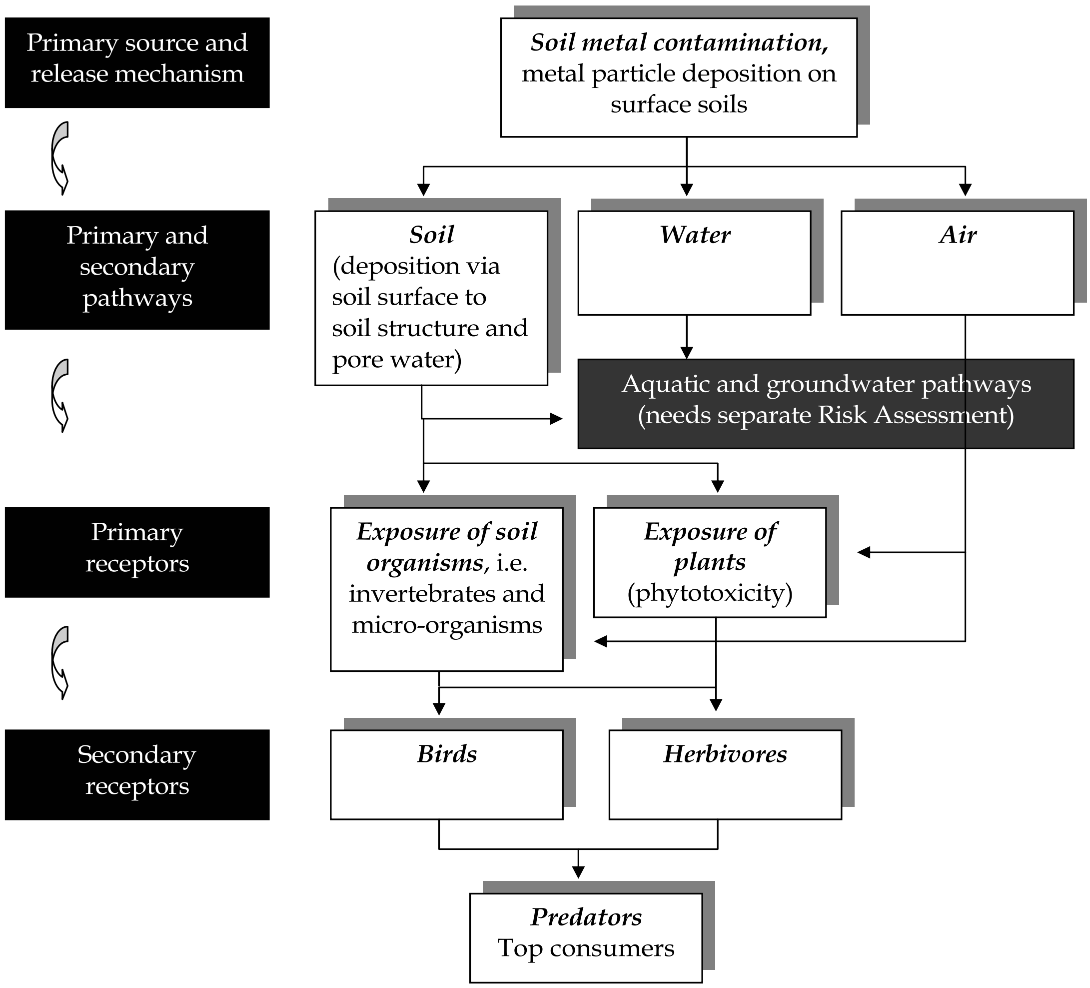

FIGURE 3 An example of a conceptual site model used to get basic information from a potentially contaminated site in Tier Zero. Adapted from Environment Agency 2003.

laboratory and/or field tests, i.e. microbial, plant, and invertebrate tests, and ecological survey data collected from the site, providing information on the survival and condition of individuals and populations of concern, are conducted as a part of the ERA (Suter et al. 2000, Environment Agency 2003). Shortly, the following steps are included in the tiered approach. Tier Zero, a rapid screening tier, establishes a robust basis for future evaluation with a conceptual site model (Fig. 3) describing what is known about the site, potential contaminants, pathways, and receptors (organism, population or community) potentially at risk. It can provide an opportunity to end ERA early, when appropriate. However, when the critical elements (contaminant – pathway – receptor) exist and potential risks are apparent, the next tier should be used. Tier One tries to use as much existing and historical information as possible to support risk management and decision making. The aim is to eliminate non-harmful substances or part of a contaminated site where risks entitle a further step and assessment. Tier One contains problem formulation, setting soil screening tools and values, selecting suitable reference sites, assessing the spatial extent of contamination, and risk characterisation. If there are no potential risks of contamination, ERA ends, and if there are certain potential risks, management actions will be started. With uncertainty, ERA should be shifted to the next tier. Tier Two, site specific characterisation, aims to survey linkages that affect the identified potential concern. It uses chemical, biological, and ecological tools for improving the quality of ERA and decreasing the uncertainties. Tier Two contains re-assessment of the problem formulation, selecting the appropriate tests and other tools for answering remaining questions and filling data gaps identified in earlier tiers, and a conclusion on whether the site is contaminated at an unacceptable level using a weight-of-evidence decision solution. In the case that all the generic and specific tests are negative without potential risks, ERA ends. If tests are positive with potential risks and without uncertainties, management actions will take place. In the presence of uncertainties, the final tier should be pursued. Tier Three aims to determine and set the likely level of exposure to contamination that has impacts on species, populations, communities, and ecosystem. It includes modelling the fate and transport of contaminant and population dynamics. To achieve these aims, model ecosystems, such as microcosm studies in the laboratory, and field experiments and monitoring should be performed (Suter et al. 2000, Environment Agency 2003).

Together with the tiered approach in ERA, it is important to consider the effects of other stressors (e.g. soil structure and land use) and environmental conditions (e.g. pH, climate change) which may impact the ecosystem and its function and which can be reflected in the ERA procedure. Consequently, the tiered approach provides a clear basis for decision making and ensures that all relevant information is taken into account when decisions concerning the site and its further management are made and most importantly, serves as a tool for preventive assessment (Suter et al. 2000).

1.6.2 Environmental conditions and soil properties – a challenge for national risk assessment

Environmental conditions in northern latitudes, such as short growing season and low temperatures with freeze-thaw cycles, are different from those in lower latitudes. These environmental conditions affect soil organisms; many species live on the edge of their distributions (Hendrix 1995, Edwards & Bohlen 1996). On the other hand, northern conditions also influence the contaminants, such as metals, affecting their fate (e.g. mobility and binding) and effects in the soil (Yaron et al. 1996, Tarradellas et al. 1997, Braunschweiler & Koivisto 2000, Allen 2002). Moreover, soil structure and properties, such as soil particles, pH, and organic matter content, are different between the latitudes, affecting soil organisms and contaminants. Differences derived from environmental conditions and soil properties can also vary considerably within the latitudes and countries. In some geological areas, it is possible that the background concentration of a substance is naturally higher than the limit of soil quality, especially in the case of some metals. Thus, it is difficult to compare the harmful concentrations of soil contaminants between the latitudes and to set mutual limit values for contaminated soil. For these reasons, countries should take into consideration their national- and even location-specific characteristics when performing risk assessment of contaminants or contaminated soils.

2 AIMS OF THE THESIS

The main purpose of this thesis was to study the harmfulness of metals to behaviour, physiology, reproduction, and growth of earthworms, and how earlier exposure to metal contaminated soil affects responses of earthworms under contamination. Moreover, the aim was to provide evidence of how responses of earthworms to metal contamination affect their functioning e.g. their role in decomposition processes. To understand earthworm responses in contaminated field soils, the study included a field survey as well as laboratory experiments, in which earthworms were simultaneously exposed to two main metal contaminants in field soils. The aim was also to find out how the results obtained in ecotoxicological experiments and standardised ecotoxicity tests can be applied in risk assessment procedures. To achieve these aims, more specifically, the following questions were set:

1) How have differently metal contaminated soils together with varying environmental conditions affected earthworm communities and metal bioavailability to earthworms along contamination gradients?
2) How do earthworms respond to metal contamination in their behaviour and physiology? Do the results from the field and laboratory experiments support each other?
3) Do earthworms from different ecological groups with different behaviour and life-history traits respond differently to metal contamination? Have e.g. tolerance to metals, metal uptake, and activity of earthworms changed during long-term metal exposure in northern soils and conditions?
4) How are responses of earthworms reflected in their interactions with other decomposers (mainly microbes) and in their role in soil processes, such as decomposition and nutrient mineralisation? Do earthworms affect metal mobility and bioavailability in the soil?

Attaining the aims will help evaluation of the usefulness of biological data from earthworms to support soil chemical analyses in quality and risk assessment of metal contaminated soils. Further, the results will also provide an answer to the question addressed by researchers and representatives from industries and regulatory bodies: What degree of earthworm responses (either behavioural, biochemical, or physiological) and alternations in earthworm communities due to soil contamination can be regarded as permissible?

3 MATERIALS AND METHODS

The materials and methods are described in more detail in the original articles (I-V, Table 1).

3.1 General view of the experiments

In addition to studying contaminated areas close to metal industries (I), four experiments in which individual earthworms from different populations and species were exposed to metals, mainly to Cu and Zn, under laboratory conditions were conducted (II-V). Cu and Zn were chosen due to determining them the main contaminants in the field measurements. A part of the experiments were performed following the standardised guidelines and a part was specially designed to answer the questions addressed in this thesis. Several parameters (such as pH, metal concentrations, amount of mineral nitrogen) were analysed from the soil samples, whereas others were measured from the earthworms; ranging from internal parameters (such as biochemical responses and metal body burdens) to population and community level responses (e.g. survival, reproduction, and decomposition activity; see Table 1).

3.2 The earthworms

Four earthworm species were used in the experiments. These species have different biological and ecological characteristics and therefore, the contribution of each species to soil processes and ecosystem functioning differ. Hereby, and also due to differences in their morphology and physiology, they will be

TABLE 1 Outline of the experiments performed in this thesis.

Article	**Experiment** (unit)	**Soil studied/used in the experiments**	**Metals studied/used in the experiments**	**Soil characteristics analysed**	**Soil metal concentrations analysed**	**Earthworms studied** (populations)	**Earthworm parameters measured**
I	Field monitoring	Clayey soil (low organic matter) Sandy soil (high organic matter)	Different metals (e.g. Cu, Zn, Pb, Fe, see I)	pH Organic matter Moisture Classification	Total and bio-available	All earthworms found	Biomass Community changes Body burdens
II	Field / Laboratory (pot)	Field soil	Simultaneously spiked Cu and Zn	pH Organic matter Moisture	Total and bio-available	*A. tuberculata* (two populations with different exposure histories)	Biochemical responses Body burdens
III	Laboratory (standardised test vessel)	Artificial soil Field soil	Simultaneously spiked Cu and Zn	pH Organic matter Moisture	Total and bio-available	*A. tuberculata* (two populations) *E. fetida*	Mortality Biomass Reproduction Avoidance Body burdens
IV	Laboratory (vessel)	Field soil Contaminated field soil	Simultaneously spiked Cu and Zn; Cu, Zn, Pb (main contaminants)	pH Organic matter Moisture	Total and bio-available	*A. tuberculata* *L. rubellus* *D. octaedra*	Avoidance
V	Laboratory (microcosm)	Field soil	Simultaneously spiked Cu and Zn	pH Organic matter Moisture Mineral nitrogen CO_2 production	Sequentially extracted	*A. tuberculata* (two populations)	Biomass Reproduction Body burdens Activity

differently exposed to harmful substances such as metals, and thus, their responses to the contamination are expected to be varied as well.

Aporrectodea tuberculata is an endogeic species and the most common and numerous earthworm in many fertile forests, agricultural and garden soils of boreal latitudes. It burrows its way through the soil taking its nutrition mainly from soil organic matter and microbes living in it. Through its burrowing and soil consuming activity, it mixes surface and deeper soil layers and has a central role in the formation of fertile mull soil structure (Edwards & Bohlen 1996).

Dendrobaena octaedra is a small epigeic species and widely distributed throughout northern Europe, having the largest variety of habitats among northern earthworms (Terhivuo 1989). It lives mainly in litter and the uppermost soil layers, taking its nutrition from humus and partly degraded plant materials. Thus, *D. octaedra* hardly mixes different soil layers with each other (Lee 1985, Edwards & Bohlen 1996).

Lumbricus rubellus is also an epigeic species, but it can, to a certain extent, mix the upper soil layers. It is common in deciduous and coniferous forests in northern Europe and more abundant in fertile soils than *D. octaedra*, taking its nutrition mainly from leaf litter (Terhivuo 1989). It has been shown that *L. rubellus* has an important role in decomposition processes and primary production in forest soil (Haimi & Einbork 1992).

Eisenia fetida is a small epigeic species that lives in habitats rich in organic matter, such as composts and dung heaps, not in natural soils as indigenous species. It is a typical r-strategists in life-history traits. Species-specific characteristics of *Eisenia*-species render them optimal for laboratory cultivation and therefore, they are standard species in ecotoxicological tests (Edwards & Bohlen 1996).

A. tuberculata was selected as the most important earthworm species in this thesis, because it has a central role in the formation of fertile mull soil. Two populations with different exposure history to metal contaminated soil were studied. The majority of the experiments in this thesis were done with *A. tuberculata* and the other species were used in certain experiments to determine species-specific differences. The standardised test species *E. fetida* was used to get essential background information for comparing its responses to metal contamination to the responses of the other species.

Earthworms were extracted from the field study areas using the formalin extraction method (I). The earthworm individuals used in the laboratory experiments were sorted by hand, except for *L. rubellus* individuals which were extracted using the formalin extraction method (II-V). The *A. tuberculata* population with long-term exposure to metal contaminated soil was collected 1 km from the Imatra Steel Oy Ab -steel smelter (Imatra, SE Finland). The soil close to the smelter contained low concentrations of different metals and the earthworm population had been exposed to the metals for generations. *A. tuberculata*, *D. octaedra*, and *L. rubellus* individuals from populations without previous exposure to metal-contaminated soil were collected in Jyväskylä, central Finland. *E. fetida* were collected from domestic compost in Jokioinen, SW Finland (Centre of Agrifood Research Finland). The collected earthworms were kept in their native soil in large containers to avoid stress, and transferred without delay to the laboratory, where the containers were stored in a climate chamber (15 °C, darkness) for a few days before the measurements and experiments began. The individuals used in the measurements and experiments were adults with well-developed clitella or subadults with clear signs of developing pubertasis (0.1-1 g in fresh mass, depending on the species).

3.3 The soils

In the laboratory experiments, deciduous forest soil rich in organic matter was collected in Jyväskylä in an attempt to find a soil having properties (other than metal concentrations) similar to the soils in which the earthworm populations and species had lived. In addition, metal contaminated field soil close (ca. 800 m) to the Cu-Ni smelter in Harjavalta (IV) and artificial soil were used in the experiments (III). Artificial soil was prepared following the standardised guideline (ISO 11268-1 1993). The field soils were homogenised by sieving through a 0.5 cm sieve before physico-chemical properties were analysed. The basis for the Cu and Zn concentrations used in the laboratory experiments were the Finnish limit values for soil quality which indicate soil contamination and need of remediation (Assmuth 1997). The soils were simultaneously spiked with appropriate amounts of Cu and Zn chlorides diluted with water so that the soil moisture content was ca. 50 % of the water holding capacity. From the metal contaminated field soil, soil concentrations were prepared by mixing the contaminated field soil with an appropriate amount of uncontaminated soil. In certain cases, if pH was low enough to affect earthworm responses during the experiment, the pH of the metal spiked and contaminated field soils was adjusted by adding and mixing appropriate amounts of calcium carbonate to the soil. The soils were mixed carefully and incubated for 7 days in a climate chamber (18 °C, darkness) to stabilise the metals in the soil before establishing the experiments.

3.4 Field study (I)

The field sampling was done along metal contamination gradients in three areas located adjacent to metal industries in Finland: a steel smelter in Imatra, a Cu-Ni smelter in Harjavalta and a Zn plant in Kokkola (Western Finland). These areas were chosen because the industries have been operating at the present locations over several decades and it could be assumed that earthworm populations from the surroundings have been exposed to metals over many generations. The emission sources have polluted the surrounding environments especially with Cu, Zn, Fe, and Pb. The emissions have been clearly decreasing at each site during the last 10 years, but metals have accumulated in the soils over several decades (Fritze et al. 1989, Derome & Lindroos 1998). Deciduous forests prevailed in the studied area around the Imatra steel smelter, whereas mixed forests were sampled in Harjavalta and Kokkola. Earthworm populations and soil samples were collected at three distances (1, 2, and 4 km) from each emission source. At all three distances, four separate plots were sampled (one plot 0.5 m^2, 12 plots from each area in total). The distance between the plots was ca. 500 m. In each area, plots were randomly chosen in similar habitats (e.g. vegetation, soil type) aiming at minimising environmental differences between sampling plots. In all the areas, the habitats sampled were dominated by silver birch (*Betula alba*) growing on mull soils.

3.5 Laboratory experiments (II-V)

3.5.1 Biomarker responses of earthworms to metal exposure (II)

Three biomarkers (concentration of metallothioneins, and cytochrome P4501A monooxygenase and glutathione-S-transferase activities) were selected for the study based on our preliminary measurements. Population samples of *A. tuberculata* were collected in the 12 plots at three distances from the vicinity of Imatra steel smelter (see 3.4). From each plot, four specimens were taken for biochemical analyses (48 totally) and four specimens were taken for metal concentration measurements (48 totally).

In addition, individuals from two *A. tuberculata* populations (with and without earlier exposure to metal-contaminated soil) were used in the laboratory experiment (totally 96 individuals from each population). The laboratory experiment was established in 192 pots (Ø=6 cm) incubated in a climate chamber (15 °C, darkness) throughout the experiment. Each pot received one worm from either of the two *A. tuberculata* populations. For both populations, pots of a control soil (without metal addition) and soils with three combined Cu/Zn concentration pairings (nominal) were established (100/175 mg kg^{-1}, 200/350 mg kg^{-1}, 400/700 mg kg^{-1} dry mass of soil). Sixteen earthworms from each population were sampled after 2, 7, and 14 days from the start of the experiment: four specimens were from the control soil and four from each soil spiked with Cu and Zn. Half of these (48 earthworms from each population) were used for measurement of metallothioneins (MT), and the other half were used for measurement of cytochrome P4501A (CYP1A) and glutathione-S-transferase (GST).

3.5.2 Standardised earthworm acute toxicity and reproduction tests completed with earthworm avoidance test (III-IV)

Acute toxicity of Cu and Zn to the earthworms was tested following the standardised guideline (ISO 11268-1 1993). In addition to the standard *E. fetida* test using artificial soil, the test was also performed with *A. tuberculata* populations with and without metal exposure history in artificial and field soils.

For the artificial soil, a control (without metal addition) and five Cu/Zn concentration pairings (nominal) from 79/138 mg kg^{-1} to 400/700 mg kg^{-1} (dry mass of soil) were prepared. The same concentration series as in the artificial soil was used in the field soil, except for one higher concentration (600 mg kg^{-1} Cu / 1050 mg kg^{-1} Zn) added to the series.

Effects of Cu and Zn on reproduction and biomass were studied using the two *A. tuberculata* populations with and without earlier exposure to metal-contaminated soil. The test was performed following the standardised guideline (ISO 11268-2 1997). However, instead of the artificial soil, field soil was used. A control soil and five different Cu/Zn concentration pairings (nominal) from 53/92 mg kg^{-1} to 267/467 mg kg^{-1} (dry mass of soil) were prepared.

The two-section earthworm avoidance tests were established in cylindrical plexiglass vessels (Ø=17 cm). Each of these vessels was divided into two equal sections using a vertical plastic divider. One half of the vessel received 500 g of control soil (section A) and the other half 500 g of contaminated soil (section B). The soil placed in section B of the respective treatment vessels contained one of the Cu/Zn concentration pairings (nominal, depending on the experiment; III, IV) from 19/32 to 300/500 mg kg^{-1} (dry mass of soil). After the divider was removed, 10 worms were placed in the resulting slit between the sections, which was then closed by gently pressing in the soil from both edges of the slit. To prevent the worms from escaping from the vessels, each vessel was securely covered with transparent cellophane having air holes for gas exchange. The vessels were kept in a climate chamber (18 ºC, darkness) for 48 h. After the test period, the divider was put back to separate the control and test soils, and the number of worms in both sections was counted. Earthworms that were cut into two parts by the reinsertion of the divider were counted as 0.5 regardless of the length of the remaining body.

3.5.3 Responses of two earthworm populations to the metals, and their effects to decomposition processes and metal availability (V)

The experiment was established in microcosms (Ø=15 cm) that received homogenised field soil which was either control soil (without metal addition) or one of the two soils simultaneously spiked with Cu and Zn (concentration pairings, 79/139 and 178/311 Cu/Zn mg kg^{-1} dry mass of soil). The experiment consisted of a main and an additional microcosm series. The main microcosm series contained 54 microcosms (36 microcosms with and 18 without earthworms), and the additional series was established in 30 microcosms (15 microcosms with and 15 without earthworms). In the main microcosm series, four earthworms from the two *A. tuberculata* populations (with and without earlier exposure to metal contaminated soil) were introduced to 18 microcosms. In the additional microcosm series, four earthworms from only the population without earlier metal exposure were added to the microcosms. The microcosms were incubated in a climate chamber (18 °C, darkness) throughout the experimental period. The main microcosm series were destructively sampled after 16 weeks. Moreover, soil and earthworm samples were taken at four weeks intervals (after 4, 8, 12, and 16 week from the beginning of the experiment) from the additional microcosms.

3.6 Analyses

3.6.1 Physico-chemical analyses

From the soil samples of the field study and the laboratory experiments, several physico-chemical properties, which may have an impact on earthworms, were measured. From each soil, pH_{H2O}, organic matter content, and moisture content were analysed (I-V). Estimated total and bioavailable (I-IV) metal concentrations were analytically determined for the soil samples. The total concentrations of metals in the soils were estimated using a rigorous ultrasound-assisted *aqua regia* extraction method and suggested bioavailable concentrations of metals were measured with an acetic acid extraction method (Streit 1984, Väisänen et al. 2002). In addition, a sequential extraction method developed by Tessier et al. (1979) was applied to describe the mobility and availability of Cu and Zn in the soils (V), and the method was advanced with ultrasound assisted extraction to decrease time and labour needed for the procedure. Mineral nitrogen forms (NH_4 and NO_3) in the soils of the microcosm experiments were analysed with a FIA-autoanalyser (Flow Injection analyser) to find out the effects of earthworms and Cu and Zn contamination on nutrient mineralisation (V).

3.6.2 Biological analyses

Earthworms were identified, counted, and weighed before and after the experiments (I-V). Effects of metals on community composition of earthworms were estimated along contamination gradients in the field areas (I). Earthworm responses to metal exposure were estimated with biochemical (II), avoidance behaviour (III-IV), biomass, survival, and offspring production changes (III, V) in the laboratory experiments. In order to get information on metal bioavailability, metal concentrations in the earthworms were analytically determined using a rigorous ultrasound-assisted *aqua regia* extraction method (I-V). Effects of the metal contamination and earthworm activity in the decomposition processes were estimated by measuring CO_2 production using an infrared carbon analyser (EQ 92 Universal Carbon Analyser; V).

3.6.3 Statistical analyses

Analysis of variance (ANOVA, significance level <0.05) was used to analyse the data derived from the field measurements and laboratory experiments. In addition, Pearson correlation coefficient and linear regressions were used to estimate differences in the parameters measured from the field (I, II). The mortality data from the acute toxicity tests were analysed with Probit analysis for LC_{50} values (III), and the soils were regarded significantly avoided by earthworms if paired-samples T-test gave significant results and if >80 % of the earthworms were found in the control soil (Hund-Rinke & Wiechering 2001),(III, IV). ANOVA for repeated measures was applied to analyse CO_2 production data (V). If ANOVA resulted in significant interactions between the main factors, these were fixed one by one and the effects of the other factor, simple effects, were analysed within the levels of the fixed factor (Zar 1999). Following ANOVA, Tukey's HSD test and two-tailed Dunnett t-test were used for pair-wise comparisons. Homogeneity of variances was tested using Levene's test, and appropriate transformation was applied to dependent variables whenever necessary. If the variances were not homogeneous even after transformations, non-parametric Kruskal-Wallis test was used instead of ANOVA. All statistical tests were performed using SPSS® software (1999), except LC_{50} values, which were analysed with Probit Analysis Program.

3.7 Instrumentation and reagents

Soil and earthworm metal concentrations were mainly measured with inductively coupled plasma (ICP) with atomic emission spectrometry (AES; Perkin-Elmer Model 2000) or with optic emission spectrometry (OES; Perkin-Elmer Optima 4300 DV). Cu concentrations in some earthworm and soil samples with concentrations too low to be measured with ICP were measured using electrothermal atomic absorption spectrometry (ETAAS, Perkin Elmer Model AAnalyst 800 with an AS-80 autosampler). All the reagents used were of analytical reagent grade and only high purity ELGA water was used. In addition, SRM (contaminated soil Standard Reference Material 2710 and 2711) samples and pure element standard solutions were regularly measured for quality control of analyses.

4 RESULTS AND DISCUSSION

4.1 Effects of metals on earthworms in the field (I, II)

Three differently metal contaminated industrial areas with different soil characteristics such as pH, organic matter and moisture contents were studied in order to render generalisations to other potentially metal contaminated areas. In Harjavalta and Kokkola, soil metal concentrations close to the emission sources were high enough to have clear harmful effects on earthworms. In Imatra, metal contamination was low and hence, its effects on earthworms were not as obvious as the effects in Harjavalta and Kokkola. It should be noticed that in all areas estimated bioavailable metal concentrations in soils were low although total soil metal concentrations were high. The results showed that responses of earthworms were mainly attributed to Cu and Zn (I).

In Harjavalta, the number of earthworm species and their biomasses decreased with decreasing distance from the smelter, and earthworms were practically lacking in the vicinity of the smelter. In addition, community structure changed along the contamination gradient; *Lumbricus* species increased with increasing distance from the smelter, whereas numbers of *D. octaedra* did not change along the contamination gradient (I). In the laboratory, *A. tuberculata* sensitively avoided the soil originating from the vicinity of the smelter (IV). The changes in earthworm behaviour and community were apparently caused by both direct toxic and indirect effects (e.g. changes in food resources and habitat quality) of metals. In Kokkola, the responses of earthworms to soil metal contamination were not as clear as in Harjavalta. Total numbers of individuals tended, however, to increase with increasing distance from the plant. Number of species was not significantly affected, possibly because the overall diversity was low even at background habitats (samples taken 4 km from the plant). In Imatra, harmful effects of metals on most earthworm parameters measured (number of individuals and species, biomass and community structure) were not observed, although soil metal concentrations were elevated close to the smelter (I). However, metal concentrations in the earthworms were high, and induction of biochemical responses (metallothioneins, cytochrome P4501A and glutathione-S-transferase) was observed in worms from populations close to the smelter reflecting early warning preceding more serious ecotoxicity (II).

It seemed that biochemical responses were mainly expressed directly due to metal exposure and increased body burdens of earthworms. However, responses were apparently also affected by indirect biochemical factors, such as effects of different inhibition processes, other than the actual metal concentration of the earthworm tissues (II). The proteins and enzymes studied take part in the detoxification metabolism of the earthworms by controlling and regulating intracellular homeostasis, thereby protecting the worms from damage caused by metal contamination (Dallinger 1993, Josephy 1997). In the field, simultaneous exposure to several metals and also other contaminants, such as certain harmful organic compounds formed in the smelting processes (Aittola et al. 1993, Wobst et al. 2003) may lead to concentration-additive effects on earthworms (Weltje 1998), although opposite results also have been reported (Khalil et al. 1996). However, in Imatra, a set of 15 polycyclic aromatic hydrocarbons (PAH) substances (US-EPA set) were analysed and their concentrations were very low and not dependent on the distance to the smelter (unpublished data). From the results obtained in the field survey, it can be concluded that biochemical responses to exposure increase earthworm tolerance and their ability to live in slightly metal contaminated habitats (I, II).

Metals are strongly bound to the soils rich in organic matter or clay (Sauvé 2002). Thus, the buffering capacity against metal contamination of the soils surveyed was apparently high; estimated bioavailable metal fractions in the soils were low compared to total metal concentrations (I). Hereby, harmful effects of metals on soil organisms were reduced. Field survey and field soils tested in the laboratory are always once-off, and it is difficult to prove the most effective element or compound for earthworm responses. Hence, exposure of earthworms and their responses to metal contamination are site-specific, depending e.g. on the composition and the amount of metal deposition and on local characteristics of the soil, vegetation and other soil organisms. Therefore, it is important to assess the harmfulness of contaminated soils site-specifically to characterise possible risks to the organisms and the functioning of the ecosystem. Earthworm data from the contaminated areas can be used as one of the biological tools, together with physico-chemical analyses, for studying the overall harmfulness of the contaminated soil.

4.2 Metal bioavailability to earthworms (I-V)

Total soil metal concentrations give general information on contaminated soils, but they have only restricted significance when assessing availability of metals to soil organisms (Allen 2002). Metal bioavailability in soils and factors determining it are not fully understood. There is no single extractable fraction simply assessing bioavailability of a given metal to all soil organisms in all circumstances (Sauvé 2002, Lanno et al. 2004). Acetic acid extractable fraction is one of the many methods suggested to be an estimate of the metal bioavailability to soil animals (Streit 1984, Morgan and Morgan 1999). However, sequential extraction procedures, using increasing strength of extraction solution, are often used to produce information for metal risk assessment (Mossop & Davidson 2003, Parat et al. 2003). Both acetic acid and sequential extraction procedures were used in the studies of this thesis to chemically estimate the bioavailability of metals to earthworms. Since bioavailability and exposure routes of earthworm metal uptake are difficult to directly determine under field conditions, earthworm internal metal concentrations are useful estimations in assessing bioavailability (Hobbelen et al. 2004).

Hardly any positive correlations between the acetic acid extractable metal concentrations in the soils and earthworm metal concentrations were found in the field. Comparisons of metal body burdens and soil metal concentrations is difficult, since a number of abiotic and biotic factors (e.g. pH, organic matter content, vegetation, and community structure of soil organisms), possible adaptation mechanisms, and general lifestyles of the species contribute to the actual exposure of individual earthworms and to their accumulation of particular metals (Morgan & Morgan 1999). Although the chemically estimated bioavailable metal concentrations did not explain earthworm concentrations better than total soil concentrations, chemical extractions clearly showed how strongly the metals were bound to the soils, giving an important view of metal mobility in the soils. Interestingly, it was observed that earthworms, together with soil aging, increased metal binding to the soils by changing metals to less mobile forms, thus evidently having an impact on the bioavailability of the metals to the soil organisms. Because the fate of metals in the soil varies in space and time, it can be concluded that their actual bioavailability to soil biota is impossible to assess without direct measuring any biological parameters, such as internal metal concentrations. Therefore, assessment of soil contamination and its environmental risks should not be based only on total metal concentrations of soils.

The ultrasound-assisted extraction method used for total metal analyses in this thesis appeared to have some advantages over formerly widely used methods such as the microwave-assisted acid leaching technique. For example, the use of an ultrasonic water bath increases sample treatment capacity, decreases sample and reagent consumption, and in addition, no special instruments are needed; the extraction can be carried out in an ordinary ultrasonic water bath. Most importantly, the ultrasound-assisted extraction procedure has proved to be repeatable with high accuracy and precision compared to standard methods (Väisänen & Suontamo 2002). Thus, the ultrasound-assisted extraction is a time-saving and cost-effective method for soil surveys.

4.3 Earthworm behavioural, biochemical, and physiological responses to metal contamination (I-V)

4.3.1 Earthworm responses to contamination

Earthworms are capable of detecting and avoiding metal contaminated soils. They clearly avoided contaminated soil at lower metal concentrations than those inducing negative responses in the standardised acute and reproduction tests (III, IV). In addition, biochemical responses measured from the individuals of the same earthworm populations and in the same soil were induced at somewhat higher soil metal concentrations than those avoided by the earthworms (II-IV). The high sensitivity of the earthworm avoidance behaviour has also been found in earlier studies with certain xenobiotics (Yeardley et al. 1996, Hund-Rinke & Wiechering 2001, Hund-Rinke et al. 2003, Schaefer 2003).

Earthworms with different ecological and morphological characteristics obviously utilise the soil environment differently, and concomitantly they have specific capabilities to avoid contaminated habitats, in addition to the fact that they are exposed to metals differently (Morgan et al. 1993, Morgan & Morgan 1999). General knowledge about earthworm characteristics, such as structure and permeability of skin, specific characteristics of chemoreceptors, trace element requirements and detoxification efficiencies, is so restricted that their consequences for earthworm behaviour cannot be reliably evaluated and quantified. Anyway, many different factors potentially affect the avoidance behaviour of earthworms, and interspecific variation in responses can be expected. Avoidance behaviour may be of crucial importance for the species populations and can significantly contribute to their exposure and survival in the field. When earthworms avoid contaminated soil, there may be potential ecological implications, even without any measurable changes in the physiology of the earthworms tested. Taking into account the important role of earthworms in soil processes, the discrimination of contaminated soil patches could lead to lowered decomposition activity and nutrient mineralisation in these soils.

The biomarkers measured, MT, CYP1A and GST, showed clear responses to metal exposure in the earthworms (II). The responses were somewhat variable and without fully consistent dose-response relations. Importantly, however, laboratory measurements largely confirmed the observations made in the field populations along the contamination gradient (Imatra), (II). Therefore, it is possible to interpret these biomarkers as indicators of soil metal contamination. The interpretation was the most consistent when all biomarkers were used in combination. Interestingly, the induction of CYP1A in the earthworms were observed under metal exposure (II), whereas earlier only a few studies have reported an induction of CYP1A in terrestrial invertebrates (Eason et al. 1998). Earlier, CYP1A in earthworms has proved to be quite difficult to measure (Liimatainen & Hänninen 1982, Milligan et al. 1986), and its activity in vertebrate models is strongly increased by several lipophilic pollutants, like PAHs and PCBs (Stegeman & Lech 1991, Stegeman et al. 1992). On the contrary, in most cases, exposure to metals inhibit the CYP1A system in vertebrates and invertebrates (Risso-de Faverney et al. 2000, Saint-Denis et al. 2001). Despite this rule, in the present study CYP1A induction was obvious due to metal contamination and appeared not to be attributed to simultaneous exposure to harmful lipophilic substances (such as PAHs and PCBs) because their concentrations were not elevated along metal gradients (Imatra). Moreover, CYP1A induction may also vary owing to many indirect effects of metal contamination, e.g. poor nutritional conditions of earthworms in metal contaminated soil may affect the induction of this part of monooxygenase system.

The disappearance of biochemical activities after a few weeks exposure in the laboratory may have been related to increased tolerance through some other detoxification mechanisms and/or phenotypic plasticity (Posthuma & van Straalen 1993). The reduced responses during exposure might also be associated to some other factors. For instance, aging of the metal contaminated soil during the experiment may lead to decreased bioavailability of metals to the earthworms already within a few weeks, as the metals become increasingly bound to the soil. Metal concentrations in the soil and in the earthworms may also have reached an equilibrium. This, in turn, could have led to levelling in the biomarker responses.

The results depicted how the earthworms responded to the soil metal contamination and how these responses were reflected at higher levels of biological organisations (I-V, Fig. 4). Summarising, earthworms first try to avoid metal contaminated soil when they face contaminated soil patches in their habitat. If they do not have possibilities to avoid metal contaminated soil (e.g. the contaminated patches are too large), biochemical responses induce. If exposure continues and biochemical detoxification systems against metal exposure are not adequate to reduce the harmful effects of metals, changes in individual activity, reproduction, and biomass appear. Finally, if exposure continues or earthworms are not able to tolerate the contamination level, the individuals will die. Changes in activity, reproduction, and survival of the individuals are reflected in the structure and functioning of the population(s) and communities, and finally in the whole ecosystem, affecting decomposition and nutrient mineralisation processes. It should be remembered that impaired reproduction and survival are not necessarily needed for changes in population and community dynamics. Also other factors, such as avoidance of patches unsuitable for living, can affect population and community dynamics.

Soil and earthworm metal concentration increases

Sub-lethal responses; Individual external sensations with chemoreceptors	Individual internal molecular responses	Individual/population responses: Changes in cocoon production and hatchlings	Functional responses; Populations and Communities	Individual/population responses; Changes in growth	Lethal responses; Individual mortality (acute toxicity)
Avoidance behaviour (III, IV)	Biochemical responses (II)	Reproduction (III, V)	Decomposition, nutrient mineralisation (V)	Biomass (III,V)	Mortality (III)
-	-	-	-	-	-
-	-	-	-	-	-
+ a,b	-	-	-	-	-
+ a,b	+ a	-	-	-	-
+ a,b	+ a	-	-	-	-
+ a,b	+ a,b	+ a	+ a	+ a,b	-
+ a,b	+ a,b	+ a,b	+ a,b	+ a,b	-
+ a,b	+ a,b	+ a,b	+ a,b	+ a,b,c	+ a
+ a,b	+ a,b	+ a,b	+ a,b	+ a,b,c	+ a,b
+ a,b	+ a,b	+ a,b	+ a,b	+ a,b,c	+ a,b,c

Sensitivity of the responses decreases

FIGURE 4 Schematic summary of the laboratory results (II-V). - = responses were not expressed, + = responses were expressed. a = *A. tuberculata* population without earlier metal exposure to metal contaminated soil; b = *A. tuberculata* population with metal exposure history; c = *E. fetida*.

4.3.2 Capability of earthworms to reduce harmful effects of metals

Earthworms are effective accumulators of metals from soils, leading to body compartmentalisation, storage, or excretion of metal ions from the most sensitive tissues or excretory organs. They apparently have well-developed, specific trafficking and storage pathways and redistribution capacity to regulate metals in their bodies, especially essential trace metals such as Cu and Zn, possibly leading to a balance between uptake and excretion (Dallinger 1993, Morgan & Morgan 1999). The physiological regulatory capacity of metals can partly explain the ability of some earthworm species to live even in highly metal contaminated areas.

In the field, earthworm body burdens seem to be site- and species-specific (I). Lock & Janssen (2001) questioned the concept of critical body burdens in assessing harmfulness of metals to earthworms in the field. High body burdens were found in all species from the study areas, also in Imatra (up to 1100 Zn mg

kg^{-1} dry mass) where contamination was the lowest (I). Similar high Zn concentrations were also measured by Morgan & Morgan (1999) and Kennette et al. (2002) from earthworms living in field soils. On the other hand, the results of the present study supported some earlier observations that body concentration of Cu is regulated in a much narrower range in earthworms than that of Zn (Marinussen et al. 1997, Morgan & Morgan 1999, Kennette et al. 2002).

High Zn concentrations in the earthworms (total body) may represent a pool of available Zn stored for future physiological demands (Simkiss 1979), since Zn participates in the control of cell respiration, and tissue growth, development, and regeneration. Zn also may have an important role in earthworms that undergo diapause when faced with unfavourable soil conditions, such as *A. tuberculata* but not *E. fetida* (Morgan et al. 1993, Morgan & Morgan 1998). On the other hand, although Cu has an important role in the transport of substances in cells and tissues, accumulation of Cu in the earthworms is low even in heavily contaminated soils (Simkiss 1979, Kennette et al. 2002). It should also be remembered that metal concentrations in the earthworms from the areas studied here were not always related to those in the soil (see 4.2). Thus, metal body burdens of earthworms should be interpreted with special care.

An additional factor that may reduce harmful effects of metals on earthworms and also increase intra-specific variation in body burdens is heterogeneous distribution of metals in the soil. For example, an earlier study demonstrated highly patchy distribution of metals in the study area at Harjavalta (Salminen & Haimi 1999). It should also be noted that, in particular, aerial metal deposition (as in all the industrial areas of the present study) may lead to an obscured pattern in species-specific accumulation (see e.g. Morgan & Morgan 1999). On the other hand, when earthworms avoid the most contaminated habitats by exploiting the less contaminated patches, they may reduce their metal body burdens and hence increase their survival in contaminated environments (Haimi & Paavola 1998, Salminen & Haimi 1999, Hund-Rinke & Wiechering 2001, III, IV).

4.3.3 Do ecologically different species have different responses to metal contamination and exposure?

D. octaedra was the only species that survived in highly contaminated areas close to the emission sources studied (especially in Harjavalta). *Lumbricus* species become dominant with decreasing contamination (I). The capability of *D. octaedra* to live in metal contaminated soils may be explained by its behavioural and life cycle characteristics, which may decrease its exposure. *D. octaedra*, more or less an annual species, lives mainly in the litter and humus layers feeding on decomposing litter material (and microbes associated with it). This litter material partly regenerates each autumn and contains much lower metal concentrations than the underlying soil organic layers (Koricheva et al. 1996, Derome & Saarsalmi 1999). *L. rubellus* and *L. terrestris*, while burrowing in the deeper soil layers, also feed on the leaf litter (Haimi & Boucelham 1991, Edwards & Bohlen 1996) and are not necessarily exposed to metals as much as e.g. *A. tuberculata*, which burrows deeper in the soil where metals have accumulated over long periods of time (Edwards & Bohlen 1996).

A. tuberculata, L. rubellus, and *D. octaedra* clearly avoided metal contaminated soil, but differences between the species were also found (IV). *D. octaedra* was the most sensitive species, avoiding already low metal concentrations, whereas *L. rubellus* responded only to the highest concentration used and was the most insensitive species studied. The differences found in avoidance responses of the species may be explained by both their behavioural and ecological characteristics (Tomlin 1992). The sensitivity of *D. octaedra* to metal contaminated soil may partly be explained by its evolutionary history in a fairly heterogeneous habitat in which environmental conditions, including the presence of harmful substances, are variable and prompt behavioural responses to changes in soil conditions may be adaptive.

L. rubellus, the least sensitive species to metal contaminated soil, burrows and spends most of its lifetime in the soil layers which are sinks for most of both natural and anthropogenic contaminants. Thus, it is possible that *L. rubellus* has adapted to higher concentrations of various compounds, including metals, and has no need to avoid moderate metal concentrations. *A. tuberculata*, continuously burrowing mainly horizontally in the soil organic layers but also deeper in the soil profile, was intermediate to the two other species in its avoidance response. Its microhabitat in the soil is presumably the most homogeneous, which may render it only a moderately metal sensitive species. In addition, there are differences between the species in many physiological and morphological characteristics, such as specific characteristics in chemoreceptors, trace element requirements, metal uptake (integument permeability to metals), excretion, and detoxification efficiencies (Stenersen et al. 1992, Morgan et al. 1993, Edwards & Bohlen 1996, Rundgren & Van Gestel 1998); all of these may contribute to specific avoidance responses.

4.3.4 Standard test species versus ecologically more relevant species in toxicity testing

E. fetida, the standard species in the acute toxicity and reproduction tests, normally found in composts and similar habitats rich in decomposing organic materials, is a typical r-strategist in its life-history traits (Haimi 1990, Edwards & Bohlen 1996). In the present study, *E. fetida* appeared to be more tolerant to metals than *A. tuberculata*: LC_{50} values were significantly higher for *E. fetida* than for *A. tuberculata* (III; see also Spurgeon et al. 2004). The lethal values for *E. fetida* were somewhat lower than those found in previous studies (Neuhauser et al. 1985, Spurgeon et al. 1994), which can be explained by the simultaneous exposure to Cu and Zn in the present study and different metal salts used in the earlier studies. Although Khalil et al. (1996) observed that simultaneous exposure to several metals can also lead to antagonistic, not necessarily to additive or synergistic, effects. Furthermore, both *E. fetida* and *E. andrei* have been found to be highly resistant to many other soil contaminants when compared to species taken from field soils (Jones & Hart 1998).

E. fetida also seemed to regulate its internal metal concentrations, especially that of Zn, at a lower level than *A. tuberculata* (III). The results support earlier observations that the body concentration of Zn in *E. fetida*, on the whole body basis, is regulated at a fairly constant level (100-200 mg kg^{-1} dry mass), regardless of the metal concentration of the soil (Lock & Janssen 2001). Tissue concentration of Cu was strictly regulated in *A. tuberculata* as well, and has also been observed in *L. rubellus* and *A. caliginosa* (Morgan & Morgan 1999). The notably higher Zn concentration in *A. tuberculata* than in *E. fetida* indicated species-specific differences in these mechanisms. Additionally, differences in the body concentrations of Cu and Zn suggested metal-specificity of the regulation mechanisms and also different metabolic requirements of these metals (Hopkin 1989). Summarising, based on the differences between the species, the choice of the species should be important in test procedures and in field surveys. Selection of an endogeic species, such as *A. tuberculata*, as a test species over the epigeic *E. fetida* gives more ecological relevance for laboratory and field bioassays in a greater number of soil types.

Inclusion of at least one additional species, i.e. *Lumbricus terrestris* Linnaeus, in these experiments might have been anticipated since it has been regarded as a proper representative species of lumbricids for ecotoxicological testing, and it has been used in many different kinds of experiments (Heimbach 1985, Slimak 1997, Stephenson et al. 1998, Paoletti 1999, Kennette et al. 2002, Shakir Hanna & Weaver 2002). However, *L. terrestris* is a large anecic species requiring more space and a deeper soil layer for its normal behaviour than usually can be offered in the tests or pot experiments in the laboratory, and therefore, it was not used in the studies (Edwards & Bohlen 1996, Nuutinen & Butt 2003).

4.3.5 Does metal exposure history matter in earthworm responses to metals?

Induction of biochemical responses in earthworms with decreasing distance to the emission source suggests that earthworms with long-term metal exposure history have better capacity to physiologically cope with metals than earthworms without earlier metal exposure. The results of the laboratory experiment supported the field observations (II). In the laboratory, the differences in the biochemical responses between the populations were fairly small, but even a slightly better capacity to e.g. detoxify metals can significantly increase the capability of an individual to live in a contaminated environment (II).

In addition to the biochemical responses of earthworms, many parameters measured (e.g. LC_{50} values, changes in biomass and reproduction) from the population with long-term metal exposure history indicated that they tolerate the metals better than the population without earlier exposure. However, no differences in the avoidance response were observed between the populations with different exposure histories (III). Biomass of earthworms without earlier exposure to metal contaminated soil increased in the control soil and in the lower Cu/Zn concentration, whereas biomass in the population with earlier exposure history did not change in either conditions. On the other hand, earthworms of the population without earlier metal exposure produced only a few or no cocoons in the control soil, but significant reproduction took place when small amounts of the metals were added to the soil (III). Maximum reproduction in the population with exposure history was found in the control soil, and cocoons were produced even in soils with higher metal concentration, at which earthworm biomass had started to decrease. Higher soil metal concentrations appeared to be harmful to the earthworms for both populations: their biomass decreased significantly and reproduction was totally inhibited (III).

Zn concentration in the earthworm tissues did not increase with increasing soil metal concentration in earthworms from the population with earlier exposure, but tended to in earthworms from the population without earlier exposure history. Earthworms with exposure history also regulated Zn at a higher level in their tissues compared to the earthworms without exposure history (III, V). Variation in tissue concentrations, however, was generally higher for earthworms from populations with earlier exposure than those without earlier metal exposure.

Interestingly, in the absence of spiked metals, the earthworms with earlier exposure to metal contaminated soil stimulated microbial activity more than the earthworms without exposure history, indicating minor differences in the function of the populations (V). In addition, the earthworms with metal exposure history seemed to decrease the mobility of metals in the soil more efficiently than the earthworms without earlier metal exposure due to earthworm activity (V).

The consistent differences between the populations, albeit not very large and not always statistically significant, imply a somewhat different capacity of the individuals to physiologically cope with metals in their habitats. Results of the experiments suggest that the population with long-term metal exposure history tolerates higher soil metal concentrations than the population without earlier exposure to metal contaminated soil. The differences between the populations seemed to be smaller in the microcosms, complicated test systems with many direct and indirect factors affecting the earthworms, than in the simplified standardised test systems. However, different allocation of resources to growth and reproduction in the microcosms may indicate adaptation to metal contamination in the population with metal exposure history. It should be noted that the pre-exposure level in the contaminated site (Imatra) was rather low (I). Therefore, the selection pressure for more efficient metal metabolism in this population is expected to have been low has been low (see also Spurgeon & Hopkin 1999). However, after careful combination and interpretation of the results, it can be concluded that earthworms from the population with earlier exposure to metal contaminated soil seem to have a better capacity to deal with metals, apparently increasing their fitness in a contaminated environment.

4.4 Effects of earthworms and metal contamination on decomposition and nutrient mineralisation (V)

Earthworms, key organisms in many ecosystems, have an important role in decomposition activity and nutrient mineralisation in the soils, especially through indirect regulation of microbial activity and community structure (Lee 1985, Haimi & Einbork 1992, Edwards & Bohlen 1996). Microcosms were used to study the responses of earthworms to metal contamination in their natural environment and to trace how these responses are reflected in important soil processes. In general, but not consistently, the presence of earthworms increased soil decomposition activity and nitrogen mineralisation. Stimulation of soil microbial activity by the earthworms was significant only in the soil with low metal concentration. The metal contamination had a greater impact on microbial activity than did the earthworms (20 % reduction from the control to the lower metal concentration and 40 % reduction from the control to the higher concentration). Earthworms slightly increased the amount of mineral nitrogen (especially NH_4) in the soil. The impact of earthworms was the highest in the soils with low metal concentration and the lowest in soils with high metal concentration (V). Thus, the results suggested that the impact of earthworms in decomposition processes manifest themselves only in moderately metal contaminated soil.

4.5 Earthworms as a tool for assessing harmfulness and possible risks of metal contaminated soils

4.5.1 Tests for studying ecotoxicity of metals and metal contaminated soils

The standardised acute toxicity test with earthworms aims to find highly contaminated soils or toxic substances. However, it offers relatively little information on the effects of low level contamination in natural environments and hardly any information on sub-lethal responses of the tested organisms although, in practice, these parameters are of crucial importance when assessing harmfulness and possible risks of contamination (Løkke & Van Gestel 1998). Reproduction and avoidance tests may provide essential data when sub-lethal effects of low level exposures and long-term ecological risks of soil contamination are considered. Sub-lethal endpoints are biologically at least as relevant as endpoints of the acute toxicity test because, in the long run, decreased reproduction and biomass and avoidance can lead to decreases in population size or even to population extinction (van Straalen & Denneman 1989). In the standardised acute toxicity and reproduction tests, the earthworms are forced to live in homogeneously contaminated test soil and they are exposed to the test substance both via their intestine and through the skin. The test procedures are somewhat laborious and time- and cost-consuming. The avoidance test procedure (standardisation is in progress) is less time-consuming and needs less testing effort than acute toxicity and reproduction tests. The major advantage of the avoidance test is that it is a sensitive laboratory bioassay that allows relatively quick and inexpensive screening of potentially toxic contaminated soils. In the avoidance test only those substances or elements (e.g. metals) which are perceived in the chemoreceptors of the test species can be detected. However, it should be noted that it is possible that earthworms may avoid concentrations of substances not having any toxic effects. For these reasons avoidance response may not be an appropriate test for use alone in risk assessment procedures.

To interpret the nature and value of the experiments used, the results should be compared to those derived from other types of experiments using the same organisms in the same soil and exposure conditions. In the present study, several experiments have been performed with the same soils, metals, and earthworm populations. When comparing the results with each other (I-V), it appears that biomass and reproduction of the earthworms were affected by the same soil metal concentrations in the microcosms and in the standardised reproduction test. On the other hand, *A. tuberculata* avoided Cu/Zn concentrations that induced no responses in the other parameters measured. Reproduction of earthworms in the microcosms decreased in similar soil Cu/Zn concentrations in which the biochemical responses of earthworms (concentration of MT, and CYP4501A and GST activities) were expressed. Because of the many, varying and uncontrolled factors in the field, it is not straightforward to link the results of the field surveys and laboratory studies. However, the studies performed showed that the same metal exposure level leads to very similar body burdens in the microcosms and in the field.

Although many tests with or without standardised protocols produce useful and comparable information on the harmful effects of contaminants on organisms and/or their functions, microcosms (and other types of model ecosystems) have a special value in ecotoxicology and risk assessment procedures (Sheppard 1997, Römbke et al. 2004, Weyers et al. 2004). Microcosms offer simultaneously information on many structural and functional parameters, rendering causal relationships between exposure and different responses to be proven. Microcosm experiments require much more time compared to short-term toxicity tests, but many potential indirect, sub-lethal, and population and community level responses, are induced only after long-term exposure (Didden 2003). Hence, microcosms offer significant and representative data when studying possible effects of contaminants or contaminated soils in both structure and functioning of ecosystems. It is important to take into account all these issues concerning different test systems when assessing possible ecological risks of contaminants or contaminated soils with biotests.

4.5.2 Standardised artificial soil versus field soils in the ecotoxicity tests

Estimated bioavailable fractions of Cu were consistently lower in the field soil than in the artificial soil, although both soils were spiked in a similar way. On the other hand, the bioavailable fractions of Zn remained very similar in both field and artificial soil. Because the amount of organic matter and pH were similar in both soils, differences may be attributed to the relative disparities in Cu and Zn chemistry in the soils and their respective interactions with the different constituents of the soils. With these notable differences between the soils, earthworms responded differently to the metal exposure in the soils. In the field soil earthworm mortality and biomass change were observed at higher soil metal concentrations than in the artificial soil (III). Although artificial soil is generally used in ecotoxicity testing, the use of field soil can be recommended as a test soil to reveal how substances behave under natural conditions.

4.5.3 Importance of earthworms as bioindicators and use of the limit values for soil quality

With careful combination of all the results, these studies show that the use of earthworms as biosensors in laboratory experiments and contaminated field sites offers relevant information about the ecological impacts of contamination in addition to soil metal analyses (see also Edwards & Bohlen 1996, Kennette et al. 2002). Analyses of metal body burdens in different earthworm species and of different soil fractions demonstrate metal bioavailability in prevailing environmental conditions. Further, analyses of earthworm populations and communities in the microcosms as a terrestrial model ecosystem and in the similar habitats along the contamination gradients in the field reveal biological effects of the contamination also at higher levels of biological organisation and decrease the gap between laboratory studies and data derived from field. Based on the results of the field survey (I), it can be noted that in Harjavalta, metal contamination close to the emission source has significantly affected the soil and earthworms. Similar effects of metal contamination have previously been found in Harjavalta also in other soil organisms (Haimi & Siira-Pietikäinen 1996, Haimi & Mätäsniemi 2002). The metals have clearly affected the functioning of the whole ecosystem at the vicinity of the emission source in Harjavalta. It is noteworth, however, that bioavailable fraction of metal concentrations were quite low even though the total concentrations were very high, indicating a good buffering capacity of the soil. The risks of contamination are high for the soil organisms close to the smelter, especially if the environmental conditions change (e.g. if there is decrease in pH). Decrease in pH may be followed by speciation of metals bound to the soil into more mobile forms, accumulation in the organisms and leaching into groundwater. In Kokkola, soil metal concentrations close to the emission source represent similar, but not as obvious, risks as in Harjavalta. In contrast to Harjavalta and Kokkola, no contamination derived harmful effects on earthworm community parameters were observed in Imatra, although metal concentrations were elevated close to the smelter. However, high metal body burdens of earthworms, induction of biochemical responses and some differences between those populations with and without earlier metal exposure history revealed harmfulness and possible risks of low level metal contamination to earthworms in Imatra (II-III, V).

In most regulatory frameworks dealing with new chemicals and products, experimental data from terrestrial ecotoxicity tests are too limited for proper ecological risk assessment. Thus, there is great demand for ecotoxicological studies on terrestrial organisms in their own habitats, and they are required to support the incomplete information on the effects of harmful contaminants to organisms. However, it should be noted that all-inclusive risk assessment will only be achieved when the responses of many different organisms (e.g. from earthworms to birds and plants) are studied.

The limit values of contaminated soils or soil quality are difficult to generalise for all organisms for all soils. Metal contamination is site-specific and hence, possible harmfulness and risks of contaminated soils to the organisms and surrounding environment are also site-specific. Therefore, the limit values for soil quality of metal contaminated soils in Finland (400 for Cu and 700 mg kg^{-1} for Zn, total concentrations) should be regarded as directive and trendsetting, when comparing the limit values to the results of this thesis. In the laboratory experiments with field soil, the Finnish limit values for Cu and Zn appeared to be too high for the earthworms; they responded already at lower concentrations and died in the limit value concentrations (II-V). On the other hand, the limit values for Cu and Zn were low for the earthworms in the field. Total concentrations measured in the field soils could be much higher than the limit values and still have no harmful effects on the earthworms (I). High total metal concentrations in the soils did reveal possible risks for soil organisms and for the environment, especially with changing conditions. Still, it is not appropriate to merely consider the total metal concentrations when assessing harmfulness and risks of metal contaminated soils. On the contrary, estimated bioavailable concentrations and the use of bioindicators and biotests as tools in assessment of possible contaminated soils would give ecologically relevant and additional information on harmfulness of the soils. Using this information we can reliably answer questions, such as: is contaminated soil really harmful to soil organisms and should we perform some remediation operation or not?

5 CONCLUSIONS

The methods applied in this thesis to measure earthworm responses to soil metal contamination appeared to be suitable for assessing harmfulness of metal contaminated soils, both in field surveys and in ecotoxicological laboratory experiments. Importantly, earthworm responses at sub-lethal range can also be used as bioassays, giving early warning signals of soil contamination. The importance in selecting a test species was emphasised, as well as the consideration of the exposure history of the population to be tested. Ecologically different species responded differently to the contamination, clearly accentuating that ecological characteristics of earthworms significantly affect their exposure and finally determine how harmful a contaminant or contaminated soil will be to them.

Earthworms seemed to have the potential to decrease mobility and hence, bioavailability of metals in the soil. The important ecological functioning of earthworms in the soil may be restricted when they avoid metal contaminated soil patches. Thus, earthworm avoidance response is an ecologically relevant endpoint. The earthworm avoidance test is a sensitive laboratory bioassay allowing quick and inexpensive screening of potentially toxic substances and contaminated soils. However, the earthworm avoidance test cannot completely replace standardised acute toxicity and reproduction tests, but it may be used to find soils needing further evaluation with standardised tests. Through its greater sensitivity to metals than standardised tests, the avoidance test may also be used to eliminate slightly contaminated soils from additional testing, thus increasing test efficiency. It is also emphasised that soil microcosm studies measure and combine various biological and chemical parameters, offering multiple and important information on harmful effects of contaminants for use in risk assessment procedures.

Considering the results of this thesis together with the observations that contamination patterns seemed to be site-specific and that metal concentrations in the earthworms were not necessarily positively correlated with soil metal concentrations, it is clear that ecological risk assessment of metal contaminated soils is challenging. The inclusion of earthworm parameters (e.g. diversity, biomass, metal body burdens) in risk assessment procedures to support chemical measurements is highly recommended. The most reliable interpretation of the impacts of metals on earthworms and on soil processes can be done when results from biological and physiochemical analyses are used in combination. When interpreted with care, earthworm data integrates the effects of metal contamination and other soil conditions on ecologically important decomposer animals. However, it is important to consider ecological characteristics and possible tolerance of the species, and even those of the population used, in the ecotoxicological tests and field surveys. Test species should be selected based on the ultimate aim of the experiment, whether it is to determine conservative screening levels, limit values for soil quality, or to perform quality and risk assessment of metal contaminated soils.

New interesting questions rose during the experiments of this thesis. More detailed information is needed on possible differences between earthworm populations with and without earlier metal exposure for species other than those studied in this thesis. While it seems that ecological and behavioural traits, as well as many physiological and morphological characteristics affect earthworm avoidance behaviour, more contributions are needed to find out the significance of avoidance behaviour to exposure of earthworms. Because it was noticed that earthworms may affect metal sorption processes in the soil, their influence on metal fate should be studied more extensively by association of the sequential extraction method in long-term exposure experiments. In addition, studies on metal mobility in soils should be extended to metal contaminated field soils in the vicinity of emission sources. The fast recovery of biochemical responses under experimental metal exposure and the lack of consistent dose-response relations also call for further experimentation for full understanding of short-term physiological responses of earthworms in metal contaminated soils and its connection to body burden via bioavailability.

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YHTEENVETO

Tässä väitöstyössä tutkittiin tärkeää, monimutkaista ja aina ajankohtaista asiaa, kuinka ihmisen toiminnasta ympäristöön päässeet haitta-aineet, esimerkkinä metallit, vaikuttavat ympäristöömme ja sen eliöihin. Metallien ympäristöhaitallisuuteen ja toksisuuteen vaikuttavat niiden kemiallisten ominaisuuksien ja pitoisuuksien lisäksi voimakkaasti myös se, mihin ne lopulta luonnossa päätyvät (esim. veteen tai maaperään) ja millaiset ominaisuudet sekä ympäristöolot tässä kohteessa vallitsevat. Useimmissa tapauksissa metallien aiheuttama ympäristön kontaminoituminen ei ole vain yhden haitta-aineen aiheuttamaa, vaan useamman aineen yhdysvaikutusta.

Maaekosysteemissä metallit päätyvät yleensä lopulta maaperään, jossa elää monimuotoinen eliöyhteisö mikrobeista hajottajaeläimiin. Kaikilla maaperän eliöillä on merkittävä osa maan rakenteen muodostumisessa, ravinteiden kierroissa ja maan kasvukunnon ylläpidossa. Eliöiden todellinen elinympäristö maaperässä vaihtelee suuresti, samoin niiden altistuminen haitta-aineille. Altistumiseen vaikuttavat haitta-aineen ja sen ominaisuuksien lisäksi myös eliön rakenne, fysiologia ja elintavat. Oleellisia maan ominaisuuksia ovat mm. pH, orgaanisen aineksen määrä, ravinteisuus, maalaji ja maannostuminen. Haitta-aineiden aiheuttamat, usein negatiiviset, muutokset maaperän hajottajayhteisössä voivat maan rakenteen ja ravinnetalouden kautta heijastua kasvillisuuteen ja siten koko ekosysteemin toimintaan.

Maaperän tilaa on arvioitu monilla menetelmillä haitallisten aineiden pitoisuuksien mittaamisesta kenttätutkimuksiin ja erilaisten eliötestien käyttöön. Eliötestit, toisin sanoen toksisuustestit, on alun perin kehitetty erilaisten yksittäisten aineiden haitallisuuden testaamiseen standardoiduissa laboratorio-oloissa. Sittemmin testejä on sovellettu myös saastuneiden maiden haitallisuuden arviointiin. Standardoiduissa testeissä mitattavana muuttujana on yleensä eliöiden kuolevuus, eräissä testeissä myös niiden kasvu ja lisääntyminen. Uusia testejä esimerkiksi maastokäyttöön ja eläinten käyttäytymisvasteiden tutkimiseen on parhaillaan kehitteillä. Näillä toksisuustesteillä ei kuitenkaan saada tietoa haitta-aineiden matalien pitoisuuksien vaikutuksista eikä pitkäaikaisvaikutuksista, jotka kuitenkin olisivat aineiden haitallisuuden, riskinarvioinnin ja ympäristönhoidon kannalta oleellista tietoa. Täten melko yksinkertaiset testit eivät välttämättä kuvaa tilannetta luonnonoloissa, eikä testituloksista voi vetää pitkälle meneviä johtopäätöksiä.

Tämän väitöskirjatyön tavoitteena oli selvittää monien lieroista mitattavien muuttujien avulla, mukaan lukien käyttäytymis- ja fysiologiset vasteet, kuinka haitallisia metallit ovat maaperässä sekä kuinka lierojen aikaisempi metallialtistushistoria vaikuttaa niiden vasteisiin altistuksen aikana. Tavoitteena oli myös yhdistää yksilö- ja populaatiotason havainnot ekosysteemin toimintaan ja tutkia, voivatko lierot vaikuttaa maaperän metallien liikkuvuuteen ja biosaatavuuteen. Eri mittausten tuloksia analysoimalla pyrittiin parantamaan laboratoriokokeiden ja kenttämittausten tulosten verrattavuutta keskenään. Lisäksi tavoitteena oli arvioida lierojen käyttömahdollisuuksia ympäristön tilan seurannassa ja riskinarvioinnissa yhdistäen maastonäytteenotto laboratoriossa tehtäviin altistuskokeisiin ja mittauksiin sekä yhdistämällä biologiset mittaustulokset kemiallisten analyysien tuloksiin.

Lieroista kerättiin näytteitä kolmelta mahdollisesti metalleilla saastuneelta alueelta eri etäisyyksiltä päästölähteistä (eri altistustasot Harjavallassa, Imatralla ja Kokkolassa). Yksilöiden lukumäärän ja yhteisön rakenteen lisäksi kerätyistä yksilöistä mitattiin useita muuttujia, kuten kehon metallipitoisuuksia ja biokemiallisia vasteita (metallotioneiini-proteiinien määrä ja sytokromi-P4501A ja glutationi-S-transferaasientsyymien aktiivisuus). Maastomittauksien lisäksi neljässä laboratoriokokeessa eri lajien ja populaatioiden yksilöitä altistettiin toistettavissa oloissa kuparille ja sinkille. Suurin osa altistuskokeista tehtiin luonnon mailla, osa keinotekoisella standardimaalla. Laboratoriokokeissa lieroista mitattiin samoja muuttujia kuin maastotutkimuksessa. Osa tämän väitöskirjan laboratoriotutkimuksista noudatti standardoituja testiohjeita, osa tutkimuslähtökohtaisesti suunniteltuja koejärjestelyitä.

Tutkimuksessa keskityttiin kolmeen pohjoisissa olosuhteissa merkittävään lierolajiin, jotka poikkeavat toisistaan niin lisääntymisbiologian, ekologian kuin metallialtistumisenkin suhteen. Metsäliero (*Dendrobaena octaedra*) on yleinen maanpinnan karikekerroksessa elävä laji, jonka lisääntyminen on käytännössä aseksuaalista. Onkiliero (*Lumbricus rubellus*) on puolestaan seksuaalisesti lisääntyvä liero, joka käyttää ravinnokseen kariketta maan pinnalta, mutta muokkaa myös maan pintaosia. Peltoliero (*Aporrectodea tuberculata*) elää syvemmällä maassa ja on tärkeä maanmuokkaaja. Oletuksena tutkimuksessa oli, että lajien erilaisella biologialla on keskeinen merkitys niiden sopeutumiseen raskasmetallialtistukseen.

Kenttätutkimukset osoittivat, että kahdella kolmesta alueesta, Harjavallassa ja Kokkolassa, maan metallipitoisuudet päästölähteiden läheisyydessä olivat haitallisen korkeita lieroille. Harjavallan kupari-nikkeli sulaton läheisyydestä lierot puuttuivat lähes kokonaan, vain joitain karikekerroksessa eläviä metsälieroyksilöitä löydettiin. Lierojen yksilö- ja lajimäärä kasvoivat selvästi päästölähteestä poispäin mentäessä. Kokkolassa lierojen vasteet maan metallipitoisuuteen eivät olleet niin selviä kuin Harjavallassa. Yksilömäärä ja lajirunsaus pieneni siirryttäessä lähemmäksi tehdasaluetta, mutta lajeja tavattiin kontrollialueillakin niukasti. Imatralla maan metallipitoisuudet lähellä metallisulattoa olivat alhaiset, eivätkä maan metallipitoisuuden vaikutukset lieroihin olleet yhtä selviä kuin Harjavallassa ja Kokkolassa. Lierojen metallikuorma oli kuitenkin suuri, ja selviä biokemiallisia vasteita havaittiin päästölähteen läheisyydessä. Kaikilla tutkituilla alueilla maan arvioidut biosaatavat metallipitoisuudet olivat varsin pieniä, vaikka kokonaispitoisuudet olivat korkeita, mikä ilmentää maiden kykyä sitoa metalleja. Tutkimuksen perusteella kupari ja sinkki olivat suurimmalta osin vastuussa lieroihin kohdistuvista haitallisista vaikutuksista.

Laboratoriokokeissa selvisi, että lierot kykenevät aistimaan ja vastaamaan metallialtistukseen jo matalissa pitoisuuksissa. Vasteet heijastuvat yksilöstä populaatioon ja yhteisön toimintaan; biokemiallisista vasteista hajotukseen ja ravinteiden vapautumiseen. Lierot välttivät jo hyvin matalia maan metallipitoisuuksia ennen kuin muut niiden vasteet ilmenivät. Lierojen altistuttua maan metallipitoisuuksille, yksilönsisäiset biokemialliset vasteet indusoituivat. Laboratoriossa tehdyt biokemialliset mittaukset tukivat maastossa tehtyjä havaintoja. Muutoksia lierojen aktiivisuudessa, lisääntymisessä ja biomassassa ilmeni puolestaan korkeammissa maan metallipitoisuuksissa kuin biokemialliset vasteet ilmenivät. Altistuminen tarpeeksi korkeissa pitoisuuksissa johti lierojen kuolemaan.

Peltolierot sietivät korkeampia maan metallipitoisuuksia luonnonmaassa kuin standardoidussa keinomaassa ennen kuin niiden altistusvasteet ilmenivät. Tunkioliero, standarditestien laji, vaikutti sietävän korkeampia maan metallipitoisuuksia ja säätelevän kehon metalleja tarkemmin kuin peltoliero. Tutkitut kolme ekologisesti erilaista lierolajia välttivät metallisaastunutta maata eritavoin, mikä osaltaan selvitti maastomittauksista löydettyjä lajikohtaisia eroja. Lajienvälisten erojen lisäksi metallialtistushistorialtaan erilaiset populaatiot vastasivat metallialtistukseen eritavoin. Aiemmin altistuneen populaation yksilöt säätelivät kehon metalleja tehokkaammin ja sietivät korkeampia maan metallipitoisuuksia kuin altistumattoman populaation yksilöt. Lierojen havaittiin vähentävän aktiivisuudellaan metallien liikkuvuutta ja biosaatavuutta maassa.

Lieroista saatujen maasto- ja laboratoriotulosten avulla voidaan yhdistää maaperän metallikontaminoituminen muiden maaperän olojen ja ominaisuuksien kanssa. Haitallisuuden arvioinnissa on kuitenkin tärkeää ottaa huomioon haitallisuuden mittarina käytettävä laji, jopa lajin populaatio. Kuten maastomittaukset osoittivat, lierojen altistuminen ja niiden vasteiden ilmentyminen metallialtistuksessa ovat paikkakohtaisia riippuen mm. päästölähteestä, paikallisista maan ominaisuuksista, kasvillisuudesta ja oloista. Tutkimus osoitti, että on erittäin tärkeää fysikaalis-kemiallisten mittausten lisäksi arvioida saastuneen maan haitallisuutta ja mahdollisia riskejä paikkakohtaisesti hyödyntäen biologisia työkaluja, kuten lieroja, riskinarvioinnissa.

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