ACCUMULATION OF METALS, COBALT (CO), MOLYBDENUM (MO) AND NICKEL (NI), TO SOIL INVERTEBRATES IN A BOREAL FOREST IN NILSIÄ, EASTERN FINLAND

Trisha Rytkönen

MSc Thesis

Environmental Biology and Biogeochemistry (EBB)

University of Eastern Finland, Department of Environmental Science

June, 2012

UNIVERSITY OF EASTERN FINLAND, Faculty of Science and Forestry, Department of Environmental Science.

Environmental Biology and Biogeochemistry (EBB)

Trisha Rytkönen: Accumulation of metals, cobalt (Co), molybdenum (Mo) and nickel (Ni), to soil invertebrates in a boreal forest in Nilsiä, Eastern Finland

MSc thesis 35 pages, 3 appendixes (3 pages)

Supervisors: Professor Toini Holopainen, PhD, Päivi Roivainen, PhD

June, 2012

Keywords: cobalt, molybdenum, nickel, metal exposure, earthworms, carabid beetles

ABSTRACT

Concerns relating to the disposal of spent nuclear fuel have elevated an interest to understand the transfer and accumulation of radionuclides in different ecosystems. Currently there are four nuclear power plants operating in Finland and they produce radioactive waste which require disposal. The first Finnish spent nuclear fuel disposal facility is planned to start operating in 2020. Ecological and environmental risks can occur if waste is not disposed of correctly. According to regulatory requirements, the disposal of spent nuclear fuel should show no detrimental effects on any plant or animal species.

The primary objective of this research was to study the transfer of cobalt (Co), molybdenum (Mo) and nickel (Ni) to earthworms and carabid beetles from soil. These are examples of elements that have radio isotopes in radioactive waste. Study area was a boreal forest in Murtolahti Village, which is located in Nilsiä, Eastern Finland. This project continues from earlier work where transfer of uranium (U) from soil to invertebrates was studied in the same area. There was some evidence that U concentrations in invertebrates were higher than in plants, and there was potential transfer of U from soil to animals.

In this study element concentrations in earthworms and carabid beetles were analysed using the inductively coupled plasma-mass spectroscopy (ICP-MS). Concentration ratios (CRs) describing the soil-to-animal transfer were calculated based on the measured concentrations.

Results showed relatively low species distribution and total number of individuals (187) for beetles. The catch of earthworms, however, was better and we collected 170 individuals. The medians of all CRs, both for earthworms and beetles, were below one suggesting that the elements studied do not transfer into earthworms and beetles very effectively. In general, the accumulation of the elements in earthworms appeared to be more likely than in beetles. However, the Mo concentrations varied highly and CRs for beetles at some plots exceeded one suggesting high soil-to-animal transfer. Therefore further studies are recommended to increase the knowledge on soil-to-animal transfer of Co, Mo and Ni.

ACKNOWLEDGMENTS

First and foremost, I want to thank my supervisors Professor Toini Holopainen and Dr Päivi Roivainen for their patience and guidance with this thesis. I am also thankful to the many people that helped me in various ways during the research process: Professor Jarmo Holopainen and Dr Anne Kasurinen for the help with the identification of the carabidae beetles and earthworm species, Dr Päivi Roivainen for the assistance in the soil sampling and thanks to Dr Hannu Koponen and Dr Anne-Marja Nerg for the guidance with the design of ecological experiments.

Warm thanks also belongs to all the staff from the Laboratory of Environmental Sciences especially Dr Minna Kivimaenpaa for her early guidance and inspiration as well as to all the helpful personnel of the Department of Environmental Science at the University of Eastern Finland.

I also want to thank my family and friends for their support in my studies and life in general. I am especially thankful to Dr Päivi Roivainen, who not only acted as my supervisor but whom worked alongside me in this research, and to my husband Dr Jani Rytkönen for his support during this journey. This thesis is for our first child, Niklas who was born in October 2008. Let’s hope one day he appreciates the endeavour made on his behalf. Mal & Ron Jenkinson (Mum & Dad) will always be the key to my heart thank you for getting me here; I will always remember your support and love.

LIST OF ABBREVIATIONS

CEC	Cation exchange capacity
Conc.	Concentration
CR	Concentration ratio
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
ICP-MS	Inductively coupled plasma-mass spectroscopy
ICRP	International Commission on Radiological Protection
OM	Organic matter
STUK	Säteilyturvakeskus (Radiation and Nuclear Safety Authority)
TC	Total concentration

TABLE OF CONTENTS

1 INTRODUCTION

Increasing environmental awareness has raised responsiveness in research to develop understanding about the transfer and accumulation of radionuclides in biological compartments of ecosystems (Yoshida et al. 2005). Radionuclide transfer in the environment can be modelled, and these models can be used to forecast the behaviour of radionuclides. Earlier models have concentrated on the radioactive fallout settings. One can observe that the most of long-lived nuclides (^{60}Co, ^{90}Sr, ^{137}Cs, ^{41}Ca, ^{59}Ni, ^{36}Cl, etc.) are accumulated in the first few years, and after that their concentration is almost a constant (Plukiene et al. 2011). Nowadays more and more spent nuclear fuel is produced and disposal facilities planned, so there is a need to study the below-ground transfer to environment in case of accidents in nuclear waste repositories.

There are four nuclear power plants in Finland. These produce different types of radioactive wastes which require disposal. Posiva Oy has submitted an application to the Finnish Government for a decision in principle on the final disposal of spent nuclear fuel waste from the existing power plants in Olkiluoto in the Municipality of Eurajoki. The disposal facility repository operation for Finland is planned to start around 2020. Ecological and environmental risks can occur if waste is not disposed of correctly. The disposal of spent nuclear fuel should cause no detrimental effects on any plant or animal species (STUK 2012). Low and intermediate radioactive wastes produced during the operation of Finnish nuclear power plants are currently disposed of at the repositories built for this purpose (Ruokola et al. 2004).

This project continues from earlier research carried out in 2007, where the transfer of several elements from soil to plants was studied (Roivainen 2011). The transfer of uranium (U) from soil to carabid beetles was also studied in 2007 (Turunen 2011). The results showed minor transfer from soil to invertebrates but U concentrations in small animals were higher than in plants.

The present study focused on the soil-to-animal transfer of cobalt (Co), molybdenum (Mo) and nickel (Ni). All of these elements have radio isotopes in different types of radioactive waste. Various species like beetles and earthworms accumulate metals and therefore are used in assessment of environmental metal pollution. The research was carried out in Murtolahti village, Nilsiä in Eastern Finland June 2008.

2 REVIEW OF LITERATURE

2.1 Soil characteristics related to element uptake

Soil is a living, dynamic ecosystem. There are several factors that affect the behaviour and bioavailability of elements in soils. The pH is a measure of acidity and alkalinity in the soil and it is considered a main variable in soils as it controls many chemical processes that take place. Most nutrient deficiencies can be avoided between the pH ranges of 5.5 to 6.5, provided that soil minerals and organic matter (OM) contain the essential nutrients for plant growth (Yoshida et al. 2005). Organic matter is made up of any plant or animal material that passes through the decomposition process and returns to the soil (Benites and Bot 2005). It is stable in the soil because it has been decomposed until it is resistant to further decomposition (Barber 1984). Organic matter is important in soil as the aggregation of binding particles helps to improve the water holding capacity as well as providing nutrients to the soil. Soils that have developed under forest vegetation usually have comparably low OM levels as less decomposition occurs (Brady 1974). Other soil properties influencing the bioavailability and solubility of metals in soil include e.g. calcium status and cation exchange capacity (CEC) as well as site condition, metal source and time of equilibration with the soil (Suthar et al. 2008; Yoshida et al. 2005). The soils OM aids nutrient availability by increasing the soil's CEC, providing chelates, and increasing the solubility of nutrients in the soil solution (Brady 1974).

The organisation of soil horizons function as a diverse community incorporating the complex interconnections between invertebrates and microbial soil ecosystems (Lavelle et al. 2006). Soil animals, such as earthworms and beetles live on the soil surface and within the soil layers, also inhabiting the natural pore systems of the soils and utilising microbes and minerals available. Concentration of metals in the soil compose an exchangeable, potentially bio-available associations (Sauvé 2002). Biotic interactions between soils and invertebrates and other soil organisms provide fundamental functions for the soil environment (Lavelle et al. 2006).

2.2 Characteristics of the study organisms

2.2.1 Carabid Beetles (Coleoptera: Adephaga)

The carabidae family makes up of over 34,000 species worldwide and most species are ground dwelling animals. They spend most of their time in the topsoil or the litter layer of the soil (Larochelle and Larivière 2007). Jelaska et al. (2011) identified that some *Carabus* species are specialised for eating earthworms and *Cychrus* species enjoy snails and slugs. Many carabid beetles are predators in soil ecosystem regulating their prey abundance. Carabid beetles are known to be main prey of many birds and terrestrial small mammals (Jelaska et al. 2011). The carabid reproductive period varies with some species being autumn breeders and having maximum adult activity late summer, or spring breeders with a max activity in spring or early summer (Jelaska et al. 2007; Jelaska et al. 2011). Carabids are usually about 1.0 - 39.0 mm in length, mostly shiny or metallic black in lustre with the wings covers (elytra). The head is narrower than the pronotum (between head and body) and has dots (slight indentations). The tarsi (end of the legs) compile five segments and the fused elytra (forewings) have deepened lines (striae) (Larochelle and Larivière 2007).

Soil ecosystem has very complex food webs, exposure pathways for inorganic metals to wild life and ecological endpoints are not always clear. Relatively little is known about the metal distribution or sites of storage in beetles (*Coleoptera)* (Kollanus 2005). Metal concentrations in carabids tissue vary between different species but the means of transport from soil to beetles as main invertebrate predators in the soil ecosystems is still unknown. However they are sensitive to their environment and they have been used as bio-indicators for these reasons (Jelaska et al. 2011). Nickel (Ni) kinetics in the ground beetle *Pterostichus oblongopunctatus* exposed to elevated temperature and fed Ni concentrated foods, showed that temperature did not affect Ni toxic kinetics in adults, but in larvae there were some temperature-dependent differences in kinetic parameters (Bednarska and Laskowski 2008).

2.2.2 Earthworms (Annelida: Oligochaeta)

Earthworms are important macrofauna in temperate and boreal ecosystems. They occupy several habitats and are found at various layers in the soil (Lee 1985). The epigeic (e.g. *Lumbricus rubellus*, *Lumbricus castaneus*) are litter dwelling species living in the organic layers of soil. The anecic (e.g. *Lumbricus terrestris*) species make a permanent living in vertical burrows (Lee 1985; Morgan and Morris 1982). They are darkly coloured at the head end (red or brown) and have paler tails. They feed on leaves from the soil surface that they drag into their burrows (Suthar et al. 2008). Endogeic (e.g. *Apporectodea rosea*) species are living in the mineral top profile of the soil (Morgan and Morgan 1999; Suthar et al. 2008). Endogeic earthworms can burrow very deeply in the soil, which makes them effective collectors of various elements in their habitat. These are often pale in colour; grey, pale pink, green or blue (Watch 2008).

Metal bioaccumulation by soil dwelling earthworms can be used as an ecological indicator of metal availability in soils (Suthar et al. 2008). Earthworms are exposed often through the skin and digestive system and recognised as a group of species able to accumulate metals (Lee 1985; Lukkari et al. 2006). These decomposer animals are relatively efficient accumulators of certain essential (Cu and Zn) and non-essential (Cd, Cu, Pb) metals (Lukkari et al. 2004; Morgan and Morgan 1999). They digest soil minerals, organic plant material as well as micro-organisms. The gut content in earthworms is regulated at a pH around neutral (Morgan and Morgan 1999) and e.g. Cu and Zn can be both passively and actively absorbed by the organism (Kollanus 2005; Lee 1985). Earthworms possess a number of mechanisms for the immobilization and excretion of metals, (such as excretion via the egesta) the relative importance of which differ between different earthworm species and metals (Peijnenburg et al. 1997).

2.3 Behaviour of elements in soil; Cobalt (Co), molybdenum (Mo), nickel (Ni)

2.3.1 Cobalt

Cobalt is naturally occurring element and it is widely distributed in rocks, soils, water and vegetation (McGrath et al. 2010). Cobalt is usually found in association with Ni showing various ranges (Table 1) between Basaltic (24-90 mg/kg**)**, Granite (1-15 mg/kg**)** igneous and shales and clays (5-25 mg/kg) (He et al. 2005). Cobalt occurs in two oxidation states (Co^{2+} and Co^{3+}). However, with the exception of certain complexes, Co^{3+} is thermodynamically unstable under typical redox and pH conditions. Cobalt is essential in trace amounts for humans and other mammals as it is an integral component of the vitamin B_{12} complex (Gal et al. 2008).

Radioactive isotopes of Co can be released to the environment during the operation of nuclear power plants. In the environment, radioactive isotopes of Co will behave chemically like stable Co. However, ^{60}Co and ^{58}Co will also undergo radioactive decay according to their respective half-lives, 5.27 years and 71 days (Gal et al. 2008). Earthworms may accumulate Co in situations where it is found in low concentrations in the environment (Gal et al. 2008)

2.3.2 Molybdenum

Molybdenum occurs naturally in soil. Background concentrations range between 0.2 and 6 mg/kg, while metal-rich soils may contain 10–100 mg Mo/kg (He et al. 2005) (Table 1). Molybdenum may be emitted to the environment by mining activities, fertilizers, and atmospheric deposition from smelters (Buekers et al. 2010).

Molybdenum is an essential element, (Vitamin B_{12}) playing a role in biochemical processes in microorganisms, plants and animals. Molybdenum is involved in enzymes that play essential roles in the biochemical cycles of N, S and P, including the reduction of nitrate, nitrogen fixation and oxidase reactions (Williams and Fraústo da Silva 2002).

The predominant dissolved species of Mo in the soil solution is the molybdate anion (MoO_4^2 with protonation taking place at low soil solution pH. The bioavailability of Mo in soil is strongly dependent on its presence as anionic species (Buekers et al. 2010). The radioactive isotope ^{93}Mo (half life 3.5 x 10^3 a) is classified as a high priority nuclide affecting the safety of spent nuclear fuel disposal in Finland (Hjerpe et al. 2010).

2.3.3 Nickel

Nickel is present in all soils, where it derives from either the parent material (lithosphere), anthropogenic deposition, or both. The concentration of Ni in most cultivated soils seldom exceeds 50 mg/kg, areas in which mafic and ultramafic bedrock is present, it can reach more than 10,000 mg/kg (Echevarria et al. 2006).

Nickel occurs in abundance in Basaltic (45-410 mg/kg**)** and Granite (2-20 mg/kg**)** igneous rocks as a free metal or as a complex with iron (Table 1) (He et al. 2005). Ni^{2+} form is stable over a wide range of pH and redox conditions prevalent in the soil. The hydrated form as Ni $(H_2O)^{2+}{}_6$, is the most common form of Ni found in the soil solution. Nickel like other metals (e.g. Cu, Zn, Fe) is an essential element for many species and deficiency and toxicity symptoms may occur when Ni is assimilated (Bednarska Ecotoxicology 2008). Anthropogenic activities additionally release Ni into the soil through sources such as metal mining and smelting, burning of fossil fuel, vehicle emissions, fertilizer application, and organic manures. Radionuclides of Ni are released to the environment in the form of Ni-63 from steel and, graphite rods Ni-59 (Yusuf et al. 2011).

Table 1 Concentration range of Co, Mo and Ni in igneous and sedimentary rocks (mg/kg). Adapted from (He et al. 2005).

Elements	Basaltic igneous	Granite igneous	Shales and clays	Limestone	Sandstone
Co	24-90	1-15	5-25	0.1	0.3
Mo	0.9-7	1-6	2.5	0.4	0.2
Ni	45-410	2-20	20-250	20	2

2.4 Ecological risk assessments

Due to a limited understanding of radiation exposure and its effects on plants and animals the International Commission on Radiological Protection (ICRP) has recommended that protection of the environment should be taken into consideration (ICRP 2007). Nuclear power plants produce spent nuclear waste at various levels; high, intermediate and low. The risk assessments look at different elements that may cause problems by mining and operating nuclear power plants such as U-235, U-238 Ni-59, Ni-63 Co-60 and Mo-93 (Plukiene et al. 2011; Plukis et al. 2008; Roivainen 2011). It is important to know how and to which extent radionuclides and other stressors may affect different organisms and therefore change community structure, distinguishing direct (toxicity) or indirect (food-chain) effects (Larsson 2004). Radioecological models are used as a tool to identify radionuclide levels to assess environment impact. Concentration ratios (CRs) between organisms and substrate are often used in these models to describe the fluxes of radionuclides. Concentration ratios simplify the complex interconnection between organisms and ecosystems. It is considered that an element accumulates to an organism when CR is higher than one (meaning that the concentration in the organism is higher than in the substrate) (Roivainen 2011).

3 AIM OF STUDY

This study was designed to provide information on the bioavailability and uptake of Co, Mo and Ni on terrestrial biota in boreal forest. The species studied, earthworms and carabid beetles, were selected to represent potential bio-indicators. Limited data on soil-animal transfer of radionuclides are available for boreal forest areas and as these forests differ from other environments it is important that more research is carried out. It is generally assumed that radionuclides act similarly to the stable isotopes of the same elements. Therefore, only stable, naturally occurring isotopes of Co, Mo and Ni were investigated in this study.

Specific aims of the study were to:

(i) Study distribution of carabid and earthworm species at the study site
(ii) Measure concentrations of Co, Mo and Ni in soil, earthworms and carabid beetles
(iii) Calculate concentrations ratios for earthworms and carabid beetles

The results of this work can be utilised in developing radioecological modelling. Knowledge obtained from the field studies will also help planning the possible further research.

4 MATERIALS AND METHODS

4.1 Site description

The sampling site (N63° 04' and E 27°54', Figs 1 and 2) was located in Nilsiä, Eastern Finland. The size of the research area was 265 m x 150 m. This site is the consequence of a small scale prospecting for uranium (U) ore in the 1960's. It was selected because U was the most important element of the larger project, this MSc project being a part of that.

Figure 1 :Dense vegetation of Nilsiä plot area

Figure 2: Vegetation and no clear understory of Nilsiä plot area

Vegetation in the area is typical to boreal forest containing deciduous and coniferous tree species (Figs 1 and 2). There is young, deciduous forest around the ore prospecting pit. Common tree species are e.g. rowan (*Sorbus aucuparia*), grey alder (*Alnus incana*) and Norway spruce (*Picea abies*) with the under-storey species composed of common wood sorrel (*Oxalis acetosella*), May lily (*Maianthemum bifolium*) and different ferns (*Dryopteris* spp., *Gymnocarpium* spp.). Spruce grows in the area where no digging was done previously and where there are less under-storey species. Thus sampling was mostly concentrated to the deciduous area.

4.2 Sampling procedures

4.2.1 Soil

A total of 40 soil samples (Table 2) were collected using a systematically designed sampling grid (1AD–10AD, Fig. 3). The first soil samples were collected with a spade on the 5th of June 2008 in light rain (Fig. 4). Carabid beetle collection pitfall traps adapted from the barber method (Gongalsky 2003) were placed on these soil sampling points. The second soil samples, total of 26 (W1 – W27, Fig. 3) were collected on the 24th of June 2008 in heavy rain. These soil sampling points were used also for the earthworm sampling. Soil samples were dried at 40 °C for two weeks, rotating soil every four days for comprehensive drying. The moisture levels were recorded from dried soil samples. The dried soil samples were sieved, and the fraction < 2 mm (sand, clay and silt) was used for the analyses of element concentrations, pH and OM content.

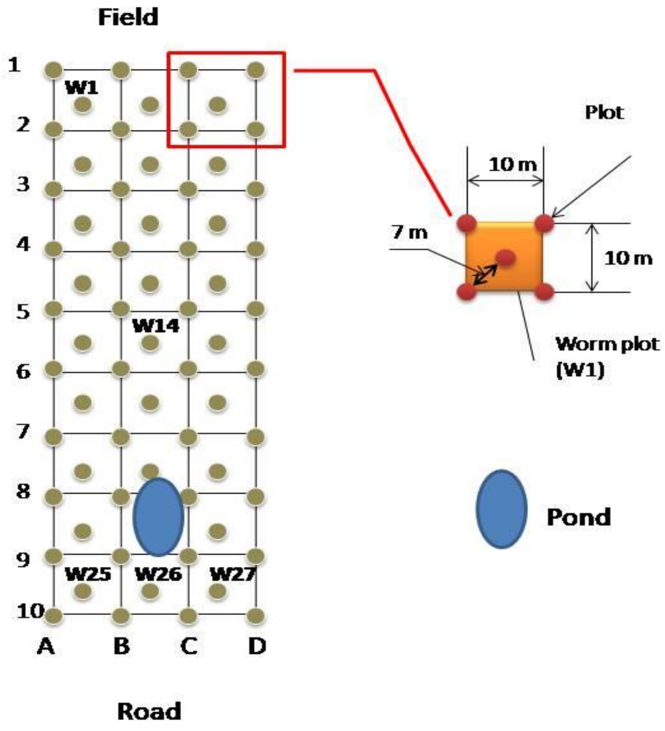

Figure 3: Organization of beetle and earthworm sampling plots. A pitfall trap for beetles was located in each corner of the squares (1AD-10AD). Earthworms were collected around the centre of each square (W1-W27).

Figure 4: Soil samples were collected with a spade on the 5th of June 2008 in light rain.

4.2.2 Carabid beetles

Pitfall traps used for beetle collection consisted of two 2 dl plastic cups covered by a white lid protecting against rainfall (Figs. 4-6). Pitfall traps contained standard coolant (50 % ethylene glycol solution) as a preservative. They were cleaned and re-applied with the coolant on a weekly basis. Species were collected and identified (Table 2) weekly between June and August.

Figure 5: Pitfall collection in coolant, two colours of coolant were used – green and blue with the same brand and consistency. The white cover had three nails in it to stabilise/attach it to the soil and to protect pitfalls from weather and potentially prevent unwanted collections of larger animals such as voles and shrews from entering the pitfall traps.

After their collection the beetles were sorted according to collection sites and frozen at -21 °C. Species identity and beetle fresh weight (FW) was then recorded before drying at 60 °C for 48 hrs. The carabids were identified using the Nikon SM2800 with C-W 10X A/22 microscope at the University of Eastern Finland Laboratory. Individual species were identified using various guides such as Chinerly (Chinerly 1973), A field guide to the insects of Britain and Northern Europe and taxonomic keys from Lindroth (Lindroth 1985; 1986). Samples were then weighed for dry weight (DW) and pooled according to these weights (to get minimum weight for chemical analyses) and collection site before being sent to the lab for metal analysis.

In total there were five pooled beetle samples and these were based on the weights (g) of the beetles. The first included beetles from plots 1AD and 2AD, the second sample from 3AB, 4AB, 5AB and 6AB, the third from 3CD and 4CD, the fourth from 7AB, 8AB, 9AB and 10AB and the fifth from 5CD, 6CD, 8CD, 9CD, 10CD (see plot locations in Fig 3).

Figure 6: The re-application of coolant in the cup of a pitfall and replacement cover for Protection of rain or other disturbances

4.2.3 Earthworms

Earthworms were collected systematically (Fig. 7) and samples taken early morning. At plots where the pond or thick tree roots and large rocks prevented sampling at the position planned, sampling was carried out in the nearest possible location. We collected 170 earthworm samples (Table 2) altogether on 24th June and the 23rd July 2008.

Figure 7: Earthworm and soil sampling in the deciduous area of the study site.

Earthworms were kept in a petri dish at room temperature to empty their gut contents onto filter paper, and the time allocated for this process was 24-48 hrs. After this the fresh weights were recorded, then earthworms were refrigerated before their size was measured and colours observed. The identification of the earthworms was done after a 70 % ethanol bath. They were identified using the Nikon SM2800 microscope with C-W 10X A/22 optics in the University of Eastern Finland. Segments and other body parts were counted, assessed and identified. Important aspects used for identification were the prostomium, clitellum and setae. Two taxonomic keys were used in the process of identification; firstly the Jyväskylä University (Anonymous 1990) and the key from Canadian earthworm watch (Watch 2008). The earthworm samples were stored in the freezer at -21 ^{o}C prior to drying at 60 ^{o}C for 48 hrs.

The pooling of earthworm samples was based on weights (g) and collection plots of samples. Seven samples were sent for analysis. The first included earthworms from W1, the second from W2, 3, 5 and 6, the third from W4 and 7 , the fourth from W8, 9, 11, 12 and 14 , the fifth from W10, 16, 19 and 22, the sixth from W18 and the seventh from W21, 24 and 27 (see plot locations in Fig. 3).

Table 2 Description of the sampling site showing beetle and earthworm samples collected 2008, including site specific information. Adapted and modified from Roivainen (Roivainen 2011).

Location	Murtolahti, Nilsiä
Coordinates	N63°04', E27°54'
Forest type	Herb-rich, *Oxalis Maianthemum*
Soil type	no clear distinction between organic and mineral layers
Range and mean of K in soil (mg kg-1)	780–4700 (1460)
Range and mean of P in soil (mg kg-1)	230–1500 (650)
Range and mean of S in soil (mg kg-1)	170–4400 (740)
Dominating plant species	May lily, narrow buckler fern, common wood sorrel, common oak fern, grey alder, rowan, Norway spruce
Number of sampling points /Beetle & Soils	40
Number of sampling points /Earthworm & Soils	26
Number of Beetles and species	182 *Carabus glabratus, Cychrus caraboides, Harpalus quadripunctatus, Harpalus rufipes, Leistus terminates, Pterostichus, Pterostichus melanarius, Pterostichus niger, Pterostichus oblongopunctatus and Pterostichus strennus.*
Number of Earthworms and species	170 *Aporrectodea spp, Aporrectodea Rosoa, Lumbricus castancus and Lumbricus terrestris.*

4.2.4 Analysis of samples

Soil pH was measured using soil-to-water volume ratio of 1:5 (device WTW inoLab pH 720 and Electrode Sentix 81). Device was calibrated using buffers at pH 4.01, 7.00, 10.01. For the analysis of soil OM content samples were kept in an oven at 550°C for three hours. The weight of each sample was measured before and after the oven. OM content equals the weight loss of the sample. Two replicates were done for every sample and the average was used as the OM value for each sample.

Element concentrations were analysed with ICP-MS (Inductively Coupled Plasma Mass Spectrometry), which is nowadays a widely used method in environmental studies. This method has remarkable advantages: detection limits (mg/kg) are low and concentrations of several elements can be measured in one analysis. The concentrations of Co, Mo and Ni in soil and animals were analysed after nitric acid digestion in a microwave oven (EPA 3051). The analyses were done in a commercial laboratory of Labtium Ltd. in Espoo, Finland. Laboratory is accredited according to the standard SFS-EN ISO/IEC 17025 (FINAS testing laboratory T025).

4.2.5 Other data used

Beetles and earthworms were collected by the same methods and from the same site also in 2007 (Turunen 2011). The locations of the pitfalls in 2007 are shown in Fig. 8. Earthworms were also collected from the same area but were only found from squares M2 and M4. The red zone highlights the U area (Turunen 2011) running through the centre of the pitfall grid. The squares M1 – M6 were in the area where sampling was done in 2008.

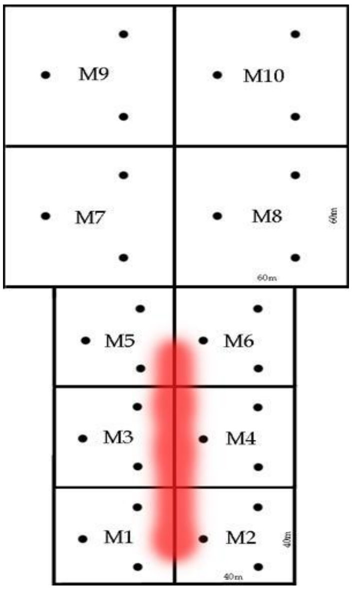

Figure 8: The beetle sampling design in 2007, the red zone highlights the uranium hotspots running through the centre of the pitfall grid. Figure adapted from Turunen (Turunen 2011).

4.2.6 Data Analysis

Concentration ratios (CR) were calculated by the equation below.

$$CR = \frac{C\ animal}{C\ soil} \qquad (1)$$

Where C_{animal} is the concentration Co, Mo, Ni in animals (beetles or earthworms) mg/kg (DW) and C_{soil} is the total concentration Co, Mo, Ni in soil mg/kg (DW).

In calculations C_{soil} is the mean of the soil element concentrations of the sampling plots pooled together.

5 RESULTS

5.1 Soil

The total concentration at individual earthworm collection plots and beetle pitfall plots displays the range from relative low values to moderately high values (Table 3). For example Mo ranged from 0.4 mg/kg at plot 6D to 60 mg/kg at plot 10D. Cobalt showed ranges (Table 3) from 2.4 -8.6 mg/kg (D.W). The total nickel concentration varied from 4.9 - 52 mg/kg (D.W).

Table 3 Median and range of the total concentration of cobalt (Co), molybdenum (Co, Mo) and nickel (Ni) in soils at earthworm and beetle sampling plots.

	Earthworm Plots		Beetle pitfall Plots	
	Median	Range	Median	Range
Co	4.1	2.4-8.6	4.0	1.4-7.2
Mo	1.5	0.4-14	1.4	0.4-60
Ni	10	5.5-29	9.4	4.9-52

The soil studied was a normal Finnish boreal forest soil with acidic pH (Table 4). High variation of OM resulted from that no clear distinction between mineral layers and organic soil (Table 4).

Table 4 Median, mean and range of pH and organic matter (OM) content in soils collected at earthworm and beetle sampling plots.

	Earthworm Plots		Beetle plots	
	pH	OM	pH	OM
median	4.6	0.1	4.6	0.1
mean	4.6	0.1	4.7	0.2
range	4.1-5.0	0.03-0.2	4.0-5.3	0.03-0.7

The contents of clay ranged from 3.6 - 18.3 and silt from 0.9 - 33.7 (Table 5). They were analysed in 2007 to obtain detailed concentrations data relating to soil to plant transfer. Clay and silt contents measured showed variation between different plots at the site (Table 5).

Table 5. Arithmetic mean values, medians and ranges of 2007 data on soil properties at the study site (n = 29). Adapted from Roivainen et al. (2011). .

Soil properties	Mean	Median	Range
Clay (%)	9.6	9.4	3.6-18.3
Silt (%)	9.8	8.5	0.9-33.7

5.2 Carabid beetles

Tab.6 shows the number of carabid species found and identified in this study in summer 2008. The identified species included: e.g. *Carabus glabratus*, *Cychrus caraboides*, *Harpalus quadripunctatus*, *Harpalus rufipes*, *Leistus terminates*, *Pterostichus melanarius*, *Pterostichus niger*, *Pterostichus oblongopunctatus* and *Pterostichus strennus.* The dominating species *Pterostichus oblongopunctatus* peaked in the mid- summer (18.6) and levelled off in the late summer. *Pterostichus melanarius* had the next highest collection at much lower numbers. Other species were levelled as catch numbers were rather low between the sampling dates (Table 6).

Table 6 Distribution of beetle species collected during 13.6 - 12.8. 2008.

Species Identified	Dates of collections summer 2008										total
	13/6	18/6	24/6	2/7	9/7	16/7	23/7	30/7	06/8	12/8	
Carabus glabratus	0	0	1	0	1	0	0	0	0	0	2
Cychrus caraboides	0	0	0	0	1	2	1	1	1	0	6
Harpalus quadripunctatus	1	1	0	3	0	1	0	0	0	2	8
Harpalus rufipes	0	0	0	0	1	0	0	0	0	0	1
Leistus terminatus	0	0	0	0	1	1	1	1	1	2	7
Pterostichus melanarius	1	5	4	5	3	2	1	3	0	2	26
Pterostichus niger	0	0	2	0	1	0	1	1	0	1	6
Pterostichus oblongopunctatus	1	49	17	25	13	6	3	1	0	0	115
Pterostichus strennus	2	5	2	0	1	0	5	0	1	0	16
	5	60	26	33	22	12	12	7	3	7	187

Beetles collected in 2007, included many of the same species as in 2008, such as the *Carabus glabratus, Cychrus caraboides, Harpalus quadripunctatus, Harpalus rufipes, Leistus terminates, Pterostichus melanarius, Pterostichus niger, Pterostichus oblongopunctatus and Pterostichus strennus.* However the total numbers of individuals collected in 2007 (617) were higher than in 2008 (187). When data was pooled some of the samples sent to analysis contained singles species due to its individual weight. Appendix 1 shows the calculated soil total concentration data of elements in soils for beetles and earthworms in 2007.

The element concentrations (mg/kg DW) of Co, Mo, and Ni in beetles are shown in Figures 9, 10 and 11. The Mo concentrations in beetles collected in 2008 (Fig. 10) were clearly higher than the concentrations in beetles collected in 2007, while no such difference was seen in the concentrations of Co and Ni at different sampling sites.

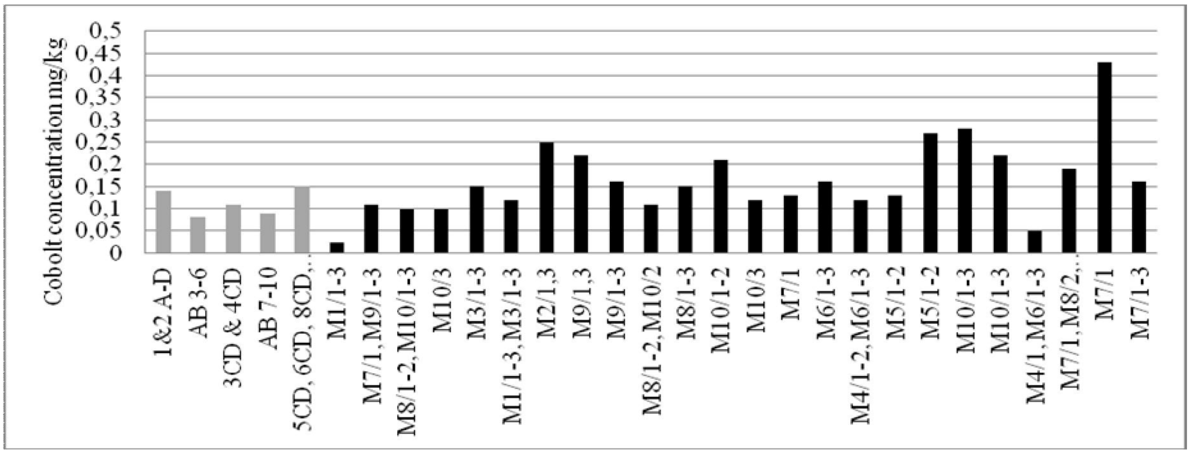

Figure 9. Cobalt concentrations (mg/kg DW) in beetles' pooled data, 2007 (black) and 2008 (grey).

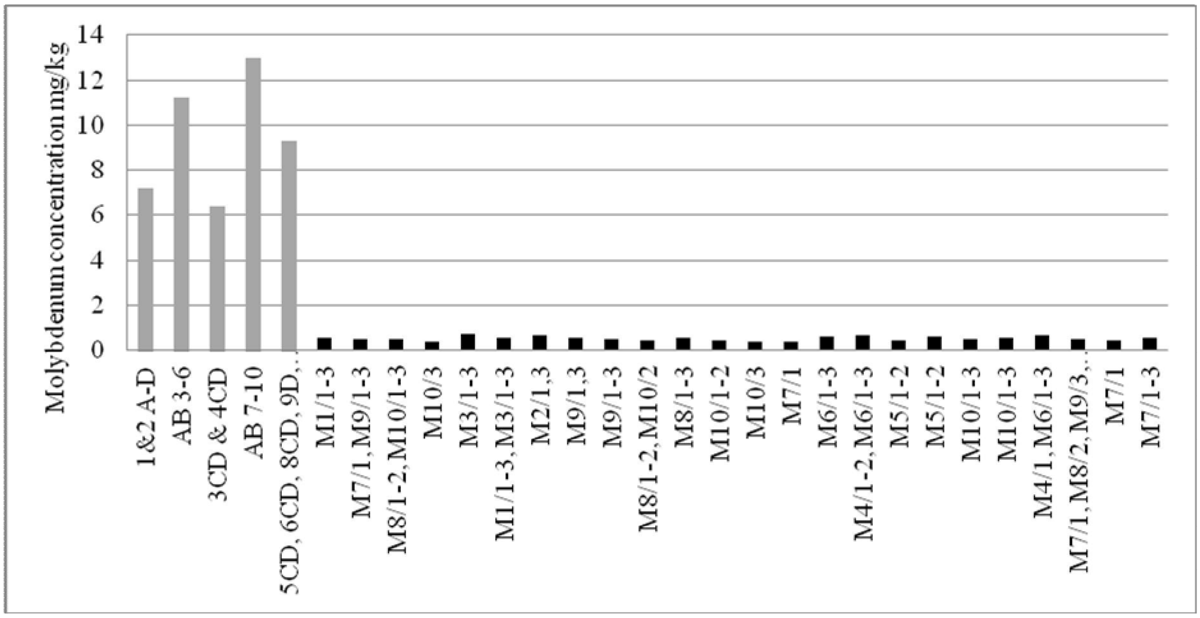

Figure 10. Molybdenum concentrations (mg/kg DW) in beetles' pooled data, 2007 (black) 2008 (grey).

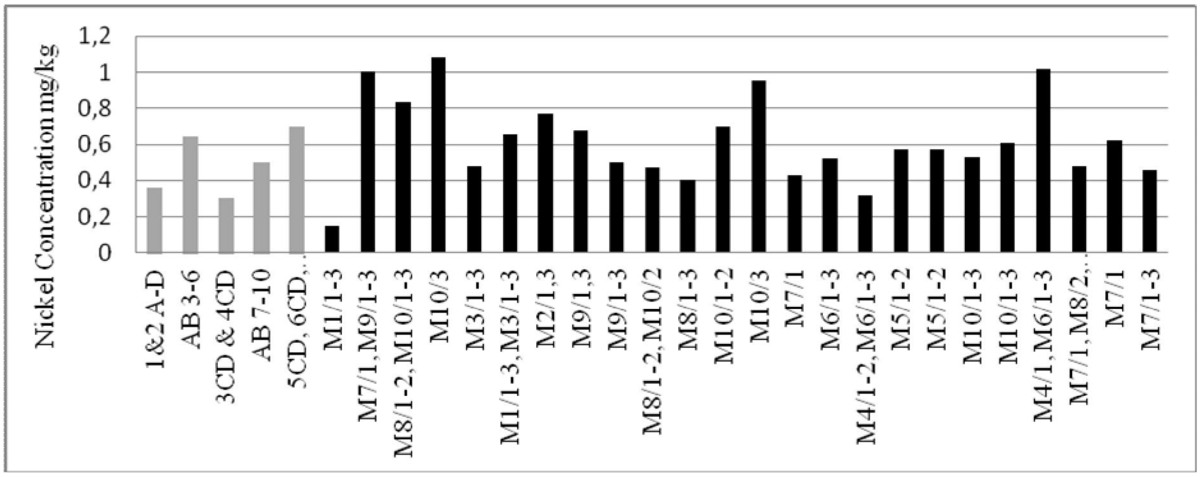

Figure 11. Nickel concentrations (mg/kg DW) in beetles in 2007 (black) and 2008 (grey).

Table 7 shows CRs for beetles. The CR values for Co and Ni were low, so there has been only low transfer. The average for Mo showed that it is above 1, meaning that there was potential transfer from soil to the beetles. However, the results for Mo were highly variable, and the median value was at low level.

Table 7 Concentration ratios of Co, Mo and Ni for beetles collected in 2007 and 2008.

	Average	Median	Min	Max
Co	0.03	0.03	0.01	0.10
Mo	1.69	0.03	0.06	17
Ni	0.05	0.03	0.02	0.11

5.3 Earthworms

The earthworm species identified included *Aporrectodea* spp, *Aporrectodea Rosoa, Lumbricus castancus* and *Lumbricus terrestris*. The concentrations of Co, Mo, and Ni were generally similar in 2007 and 2008 (Fig 13). Cobalt was the element that showed highest variation range from less than 2 mg/kg at plot W18 and 8 mg/kg at plot W1.

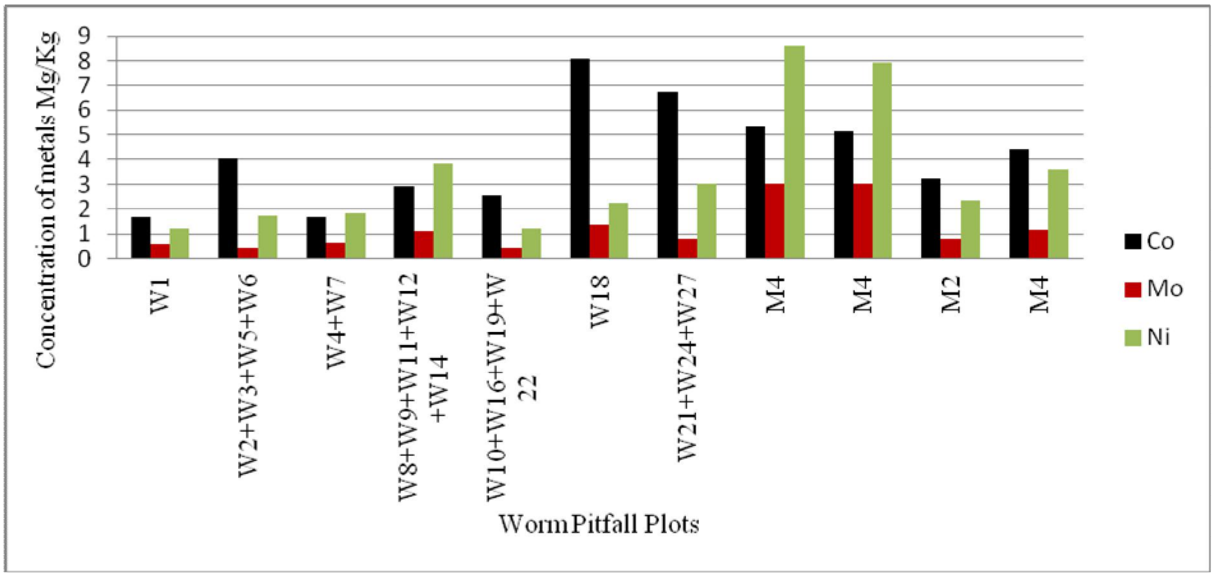

Figure 12 Total metal concentrations (Co, Mo and Ni) in earthworm's pooled data 2008 labelled W1 to W27 and 2007 labelled M2 and M4

The CRs for earthworms were highest in Co (Table 8). However, all the CRs were quite low suggesting that transfer of these elements from soil to earthworms has not been high. The average CR for Co was 1.02 indicating potential transfer of this element.

Table 8 Concentration ratios for earthworms collected in 2007 and 2008.

	Average	Median	Min	Max
Co	1.02	0.98	0.40	2.5
Mo	0.68	0.58	0.08	1.55
Ni	0.36	0.20	0.10	1.04

6 DISCUSSION

The low catch of beetles in 2008, compared to the previous year of 2007 was probably due to cool and wet summer. The summer in 2007 was a more typical Finnish summer with much less rain fall. The moderately low numbers of carabid species found and identified in this study resembled effects of decreasing temperature on carabids (Bednarska and Laskowski 2008). The sampling dates clearly affected the distribution for *Pterostichus oblongopunctatus* as it peaked in early summer and tethered off mid to late summer, and similar occurrences were seen for *Pterostichus melanarius* (Fig. 9). However other species numbers were similar and catch information had not differentiated a great deal between the sampling dates (Fig. 9)

Ground beetles are important in biological control, nutrient cycling, and linking above and below ground ecological systems (Lövei 2008). Transport of contaminants in ecosystem may occur through food webs (Jelaska et al. 2011). However, if methodology – such as collection time and frequency was influenced by animals' habitats exposing them to higher or lower limits at various stages of their lifecycle and time of analysis, it could well have affected species distribution and collection. This can suggest we should have emptied pitfalls more often. Another suggestion to improve pitfall collections is to set up pitfalls earlier in the summer to account for earlier lifecycle stages. Pitfalls remain suitable for studying various population parameters such as species presence and species abundance (Lövei and Sunderland 1996). The identification process in this study could have also included differentiation of carabid beetles between sexes as other studies show that females carry higher element concentrations (Jelaska et al. 2007).

The metal bioavailability and total concentrations of earthworms and beetles was evaluated to assess transfer. Soil concentrations were generally within the background range of elements (Roivainen, 2011). However, adjacent plots showed soil concentrations ranging from high element concentration to very low element concentrations. This could have been misleading in figures and tables with pooled data. The effect of pooling could have distorted the true distribution of metals at the habitat of beetles and earthworms. Especially the concentrations of Mo were highly variable, both in soils and animals. Some plots had very high soil Mo concentrations. Molybdenum was the element for which the highest CR values in beetles were found and therefore it is possible that it accumulates into beetles. Molybdenum is least soluble in acid soils, like the soil in this study, and readily mobilised in alkaline soils (Roivainen et al. 2011). Therefore it is possible that soil-to-animal CRs are even higher at more alkaline conditions. Cobalt concentrations for bioaccumulation are subjective to soil water status and seasonality all of which influence the uptake and retention of pollutants in the soil (Gal et al. 2008). Nickel concentrations show relatively low results in comparison with the pooled data. However, the high variation in concentrations made it hard to draw conclusions, and further studies are recommended.

The elements selected showed relevance for ecological risk assessments related to boreal forests. The environmental importance and exposure contamination in this study and in literature (Jelaska et al. 2011; Lövei 2008; Lukkari et al. 2004) showed it is significant to consider these areas further. The accumulation of the elements in earthworms appeared to be more likely than in beetles. This could be attributed to habitat range where earthworms are more exposed (Table 8). The difference in burrowing patterns of earthworms can influence the patterns of metal bioaccumulations between endogeic and anecic species, and therefore CRs obtained here may not be suitable for all species. Other factors such as pH and OM are also contributory (Suthar et al. 2008). Characteristics of radionuclides are important for long term changes in ecosystems. Identification of element concentrations and calculation of CRs, both for earthworms and beetles, contribute to identifying complex ecological relationships in terrestrial ecosystems.

7 CONCLUSION

In the present study concentrations of the elements Co, Mo and Ni were measured in soils, earthworms and carabid beetles and CR values describing soil-to-animal transfer were calculated. Overall, earthworms had higher CRs than carabid beetles and that is probably due to habitat where earthworms are more exposed. Concentration ratios were generally low suggesting that the elements studied do not accumulate to earthworms and beetles. However there was variation in concentrations and CRs, especially in the case of Mo. Therefore further studies are recommended to increase the knowledge on soil-to-animal transfer of Co, Mo and Ni. Methodology used in this study was prosperous and transferable to other research.

REFERENCES

Anonymous 1990 Ecological and Environmental Science key from Jyväskylä University, Jyväskylä

Barber S A 1984 Soil Nutrient Bioavailability: A Mechanistic Approach. Wiley, New York.

Bednarska A and Laskowski R 2008 Effects of nickel and temperature on the ground beetle *Pterostichus oblongopunctatus* (Coleoptera: Carabidae). Ecotoxicology 17, 189-198.

Benites J and Bot A 2005 The importance of soil organic matter, Key to drought-resistant soil and sustained food production. FAO Journal Soil Bulletin 80.

Brady N C 1974 The Nature and Properties of Soils. Macmillan Publishing Co., New York.

Buekers J, Mertens J and Smolders E 2010 Toxicity of the molybdate anion in soil is partially explained by effects of the accompanying cation or by soil pH. Environmental Toxicology and Chemistry 29, 1274-1278.

Chinerly M 1973 A Field Guide to Insects of Britain and northern Europe. Collins, London.

Echevarria G, Massoura S T, Sterckeman T, Becquer T, Schwartz C and Morel J L 2006 Assessment and control of the bioavailability of nickel in soils. Environmental Toxicology and Chemistry 25, 643-651.

Gal J, Hursthouse A, Tatner P, Stewart F and Welton R 2008 Cobalt and secondary poisoning in the terrestrial food chain: Data review and research gaps to support risk assessment. Environ. Int. 34, 821-838.

Gongalsky K B 2003 Impact of pollution caused by uranium production on soil macrofauna. Environmental Monitoring and Assessment 89, 197-219.

He Z L, Yang X E and Stoffella P J 2005 Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology 19, 125-140.

Hjerpe T, Ikonen A K T and Broed R 2010 Bioshpere assessment report 2009. Report 2010-3. Posiva Oy, Olkiluoto.

ICRP 2007 The 2007 Recommendations of the International Commission on Radiological Protection. ICPR Publication 103. Annals of ICRP 37.

Jelaska L S, Blanusa M, Durbesic P and Jelaska S D 2007 Heavy metal concentrations in ground beetles, leaf litter, and soil of a forest ecosystem. Ecotoxicology and Environmental Safety 66, 74-81.

Jelaska L S, Jurasovic J, Brown D and Symondson W 2011 Prey Detection by Molecular Analyses of Gut-Content in Carabid Beetles and Metal transfer from Soil to Beetles Through Food Web. In 15th European Carabidologists Meeting, Daugavpils, Latvia 23.-27.8.2011.

Kollanus V L 2005 Uptake and Effects of Chromium and Multiple Metal Contaminants in Terrestial Biota in the Vicinity of Stainless Steel Works. University of Kuopio.

Larochelle A and Larivière M-C 2007 Fauna of New Zealand, Ko te Aitanga Pepeke o Aotearoa, Carabidae (Insecta: Coleoptera): catalogue Number 43. Whenua Press, Manaaki.

Larsson C M 2004 The FASSET framework for assessment of environmental impact of ionising radiation in European ecosystems - an overview. Journal of Radiological Protection 24, A1-A12.

Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P and Rossi J P 2006 Soil invertebrates and ecosystem services. Eur. J. Soil Biol. 42, S3-S15.

Lee K E 1985 Earthworms. Their ecology and relationships with soils and land use. Academic Press, Sydney.

Lindroth C M 1985 The Carabidae (Coleoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavia 15.

Lindroth C M 1986 The Carabidae (Coleoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavia 15.

Lövei G L 2008 Ecology and conservation biology of ground beetles (Coleoptera: Carabidae) in an age of increasing human dominance.

Lövei G L and Sunderland K D 1996 Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology 41, 231-256.

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

Lukkari T, Teno S, Vaisanen A and Haimi J 2006 Effects of earthworms on decomposition and metal availability in contaminated soil: Microcosm studies of populations with different exposure histories. Soil Biology and Biochemistry 38, 359-370.

McGrath S P, Mico C, Curdy R and Zhao F J 2010 Predicting molybdenum toxicity to higher plants: Influence of soil properties. Environmental Pollution 158, 3095-3102.

Morgan A J and Morris B 1982 The accumulation and intracellular compartmentation of cadmium, lead, zinc and calcium in two earthworm species (Dendrobaena rubida and Lumbricus rubellus) living in highly contaminated soil. Histochemistry 75, 269-285.

Morgan J E and Morgan A J 1999 The accumulation of metals (Cd, Cu, Pb, Zn and Ca) by two ecologically contrasting earthworm species (Lumbricus rubellus and Aporrectodea caliginosa): implications for ecotoxicological testing. Applied Soil Ecology 13, 9-20.

Peijnenburg W J G M, Posthuma L, Eijsackers H J P and Allen H E 1997 A Conceptual Framework for Implementation of Bioavailability of Metals for Environmental Management Purposes. Ecotoxicology and Environmental Safety 37, 163-172.

Plukiene R, Plukis A, Puzas A, Remeikis V, Duskesas G and Germanas D 2011 Modelling of Impurity Activation in the RBMK Reactor Graphite
Using MCNPX Progress in Nuclear Science and Technology 2, 421-426.

Plukis A, Remeikis V, Juodis L, Plukiene R, Lukauskas D and Gudelis D 2008 Analysis of Nuclide content in Ingalina NPP radioactive waste streams. Lithuanian Journal of Physics 48, 375-379.

Roivainen P 2011 Characteristics of soil to plant transfer of elements relevant to radioactive waste in boreal forest. In Dissertation in Forestry and Natural Sciences 056. University of Eastern Finland.

Roivainen P, Makkonen S, Holopainen T and Juutilainen J 2011 Soil-to-plant transfer of uranium and its distribution between plant parts in four boreal forest species. Boreal Environment Research 16, 158-166.

Ruokola E, Eloranta E, Hutri K-L and Tikkinen J 2004 Radioaktiiviset jätteet. In: Sandberg J (ed.) Ydinturvallisuus (in Finnish). Radiation and Nuclear Safety Authority, Pages 269-318, Helsinki.

Sauvé S 2002 Speciation of Metals in Soil; In Allen, Herbert E.(ED) Bioavailability Of Metals in Terrestrial Ecosystems: Importance in partitioning for bioavailability to invertebrates, microbes and plants. Society of Environmental Toxicology and Chemistry (SETAC), Pensicola, FL. pp. 7-38.

STUK 2012 Website of Radiation and Nuclear Safety Authority, Accessed 4.4.2012 www.stuk.fi.

Suthar S, Singh S and Dhawan S 2008 Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category? Ecological Engineering 32, 99-107.

Turunen S P 2011 Pohjois-Savon uraaniesiintymilla Elavien Maapera- ja kenttakerroksen Elainten Uraapitoisuudet. In Ymparistotieteen laitos. University of Eastern Finland.

Watch W 2008 Key to Productively Mature Earthworms Found in Canada.

Williams R J P and Fraústo da Silva J J R 2002 The involvement of molybdenum in life. Biochemical and Biophysical Research Communications 292, 293-299.

Yoshida H, Muramatsu Y and Peinjnenburg W J G M 2005 Multi-element analyses of earthworms for radioecology and ecotoxicology. Radioprotection 40, S491-S495.

Yusuf M, Fariduddin Q, Hayat S and Ahmad A 2011 Nickel: An Overview of Uptake, Essentiality and Toxicity in Plants. Bull. Environ. Contam. Toxicol. 86, 1-17.

APPENDIX 1

Table 9 2007 Calculated soil to concentration (mg/kg) DW data

Sample Plot	Co	Mo	Ni
	mg/kg	mg/kg	mg/kg
	Soil conc.	Soil conc.	Soil conc.
earthworm, M4	4.08	1.97	8.21
earthworm, M4	4.08	1.97	8.21
earthworm, M2	5.10	10.51	13.67
earthworm, M4	4.08	1.97	8.21
beetle, C. caraboides M1	3.91	0.39	6.58
beetle, P. oblongopunctatus M7, M9	5.46	1.97	11.53
beetle, P. oblongopunctatus M8, M10	4.25	0.65	9.13
beetle, P. niger M10	6.45	0.38	13.50
beetle, Pterostichus M3	3.46	0.46	6.52
beetle, Pterostichus M1, M3	4.71	0.77	9.45
beetle, Pterostichus M2	5.10	10.51	13.67
beetle, P. niger M9	6.38	2.93	10.45
beetle, Pterostichus M9	6.38	2.93	10.45
beetle, P. niger M8, M10	4.25	0.65	9.13
beetle, P. niger M8	2.78	0.83	6.22
beetle, P. niger M10	6.45	0.38	13.50
beetle, P. niger M10	6.45	0.38	13.50
beetle, P. niger M7	4.54	1.01	12.60
beetle, P. niger M6	3.93	1.38	9.96
beetle, Pterostichus M4, M10	5.03	1.33	10.32
beetle, Pterostichus M5	6.60	5.17	19.77
beetle, Pterostichus M5	6.60	5.17	19.77
beetle, Pterostichus M10	6.45	0.38	13.50
beetle, Pterostichus M10	6.45	0.38	13.50
beetle, C. caraboides M4, M6	4.01	1.68	9.09
beetle, C. glabratus M7, M8, M9, M10	4.91	1.37	10.44
beetle, P. niger M7	4.54	1.01	12.60
beetle, Pterostichus M7	4.54	1.01	12.60

APPENDIX 2

Table 10 and Table 11 2008 data; Total concentration (mg/kg)

2008 Total concentration (mg/kg) DW beetles soil data			
	Co	Mo	Ni
1A	6.60	0.72	9.93
1B	5.70	0.82	8.09
1C	4.10	2.55	9.62
1D	3.55	0.91	8.68
2A	4.28	0.78	7.89
2B	4.72	0.41	9.33
2C	7.24	3.54	17.2
2D	3.23	1.12	7.62
3A	3.75	0.76	7.75
3B	6.70	2.00	15.6
3C	3.46	1.36	8.12
3D	4.43	2.07	9.38
4A	4.00	0.68	7.99
4B	6.85	1.21	17.5
4C	4.14	2.57	17.6
4D	4.59	1.44	9.47
5A	3.98	0.53	7.87
5B	1.95	2.06	7.74
5C	5.30	2.32	14.7
5D	1.37	2.35	7.03
6A	3.34	0.61	7.11
6B	3.57	4.35	11.2
6C	3.63	7.30	18.2
6D	5.03	59.6	21.1
7A	1.53	1.02	8.22
7B	1.94	0.29	4.86
7C	4.01	1.86	9.38
7D	1.57	5.04	8.95
8A	3.17	0.69	7.53
8B	2.61	0.45	6.06
8C	3.43	0.40	7.70
8D	10.3	13.3	13.3
9A	2.41	1.30	10.9
9B	5.30	0.40	10.6
9C	6.51	1.73	19.3
9D	3.28	2.28	9.94
10A	2.78	1.40	10.5
10B	6.08	0.69	11.6
10C	5.09	9.30	16.3
10D	5.92	7.09	51.8

2008 Total concentration (mg/kg) DW earthworms soil data			
	Co	Mo	Ni
W1	4.00	0.66	7.85
W2	4.84	0.69	8.99
W3	3.92	2.13	10.5
W4	4.13	0.59	7.44
W5	3.79	1.06	12.2
W6	4.01	1.19	9.01
W7	4.14	0.60	8.62
W8	8.09	4.30	29.4
W9	4.46	3.21	12.5
W10	3.99	0.57	8.16
W11	7.22	5.20	24.3
W12	3.64	1.16	8.13
W14	7.44	6.36	24.2
W16	2.70	0.41	5.51
W18	2.73	2.38	9.32
W19	3.36	2.06	9.78
W21	8.57	13.6	19.3
W22	6.98	0.67	23.8
W24	4.18	1.74	9.98
W27	5.60	1.89	15.0

APPENDIX 3

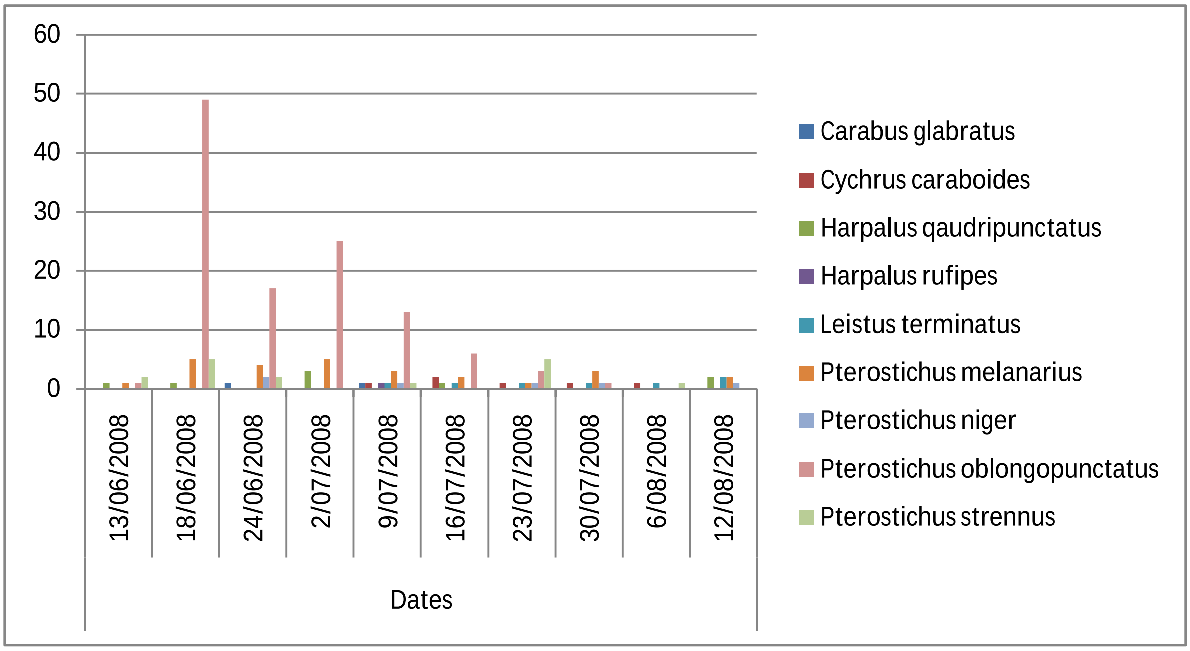

Figure 13: Distribution of beetle species collected during 13.6 - 12.8. 2008.