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Uranium and toxic metal uptake by the earthworm *Eisenia hortensis* in contaminated soils

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Radioecology

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Preface

This study was performed under the umbrella of the MINEXRIM project (Mineral extraction in the High North – Radiological Risks, Impacts and Mitigation) and was performed in collaboration with the Norwegian Radiation Protection Authority (NRPA) and the Norwegian Institute for Water Research (NIVA). The work is the final thesis presented in the fulfillment of the criteria for a two-year Master of Science degree in Radioecology at the Norwegian University of Life Sciences, at the Centre for Environmental Radioactivity (CERAD).

I would like to thank my lead supervisor Prof. Lindis Skipperud (NMBU/CERAD) and my co-supervisors Frøydis Meen Wærsted (NMBU/CERAD), Louise Kiel Jensen (NRPA) and Prof. Deborah Oughton (NMBU/CERAD) for allowing me to be a part of the MINEXRIM project and for their guidance, kindness and support throughout this work. I would also like to thank Marit Pettersen, Karl Andreas Jensen, and Solfrid Lohne at NMBU for their guidance in the laboratory and in particular with ICP-MS analyses. A special thanks goes to Lene Valle, for performing the ICP-MS analysis of all 200+ earthworm and soil samples at NMBU. I also wish to thank Emmanuel Lapied for invaluable discussions on all matter earthworms, and Lisa Rossbach for advice in designing my experiment.

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Most importantly, I want to thank my family for their ever present support – to my parents and sister for their constant encouragement and to my wife and son (who appeared halfway through this course of study) for their understanding and unwavering support.

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Summary

Earthworms are present in nearly every terrestrial ecosystem worldwide and play an important role in the transport and availability of nutrients and other elements in soil. Even more ubiquitous than earthworms are naturally occurring radioactive material (NORM) and toxic metals – elements that are present in all soils, albeit in widely varying concentrations. These elements often can be found in a bound state (e.g. within a rock matrix), but can as a result of both natural and anthropogenic processes (e.g. mining and construction, wood burning, weathering) become more readily accessible for uptake in living organisms.

The aim of this study was to explore uptake of Mg, K, S, Zn, Cu, Cr, Mn, Co, Mo, V, Ni, As, Cd, Sn, Pb and U in varying degrees of contaminated soil by the earthworm species *Eisenia hortensis* in a laboratory setting. Furthermore, we wanted to compare uptake observed in the laboratory to uptake in earthworms sampled in the same soil as used in one of the laboratory treatments, chosen for its known high natural uranium (U) concentration. A control soil was collected from an area with low concentrations of NORM and toxic metals (Romeriksåsen), and the soil from the high U region (Orrefjell) was mixed with alum shale from Gran, Hadeland, to give a gradient of toxic metals and a stable high U concentration.

It was shown that increasing the metal concentration while maintaining a stable high U concentration had no effect on the assimilation of U by *E. hortensis*. In addition, increased mortality and decreased reproductive health were observed at the highest concentrations of metals tested, while at lower levels a positive effect on reproductive health was observed. Metal and NORM uptake in laboratory and field-sampled earthworms in the same soil was constant for most elements, with an increased concentration in laboratory earthworms for Co and Pb.

Sammendrag

Meitemark er å finne i de fleste terrestriske økosystemer og er viktige for transport og tilgjenglighet av næringsstoffer og andre grunnstoffer i jord. Enda mer utbredt enn meitemark er «naturlig forekommende radioaktivt materiale (NORM)» og giftige metaller - grunnstoffer som finnes i alle typer jord, men i vidt forskjellige konsentrasjoner. Disse grunnstoffene er ofte sterkt bundet i mineraler eller andre matriser i jord, men kan bli mer tilgjengelige som resultat av menneskelige og naturlige prosesser (for eksempel gruvedrift, bygg- og annleggsvirksomhet, forbrenning av fossilt materiale, forvitring).

Målet av denne studien var å undersøke opptak av Mg, K, S, Zn, Cu, Cr, Mn, Co, Mo, V, Ni, As, Cd, Sn, Pb og U fra jord kontaminert i ulik grad til meitemarkarten *Eisenia hortensis* i laboratorieforsøk. I tillegg var det ønskelig å sammenligne opptak observert i laboratoriet med opptak i mark prøvetatt i felt i samme jord – jord som ble valgt på grunn av kjent høy urankonsentrasjon. Kontrolljord ble hentet fra et område med lave konsentrajoner av NORM og giftige metaller (Romeriksåsen), og jord med høyt uraninnhold (Orrefjell) ble blandet sammen med alunskifer fra Gran (Hadeland) for å skape en gradient av giftige metaller og en stabil høy urankonsentrasjon.

Økende metallkonsentrasjoner i jord med en stabil høy urankonsentrasjon hadde ingen påvirkning på opptak av uran i *E. Hortensis*. Økt dødlighet og lavere reproduksjon ble observert i jorden med høyest innhold av metaller, mens i jorden med middels innhold av metaller ble det observert en positiv effekt på reproduktivitet. Opptak av metaller og NORM i laboratorie- og feltprøver var tilnærmet likt for de fleste grunnstoffer, med unntak av Co og Pb som ble tatt opp i større grad av mark i laboratorieforsøket.

1 Introduction

One of the major environmental challenges of our time is the spreading of both man-made and natural harmful compounds, and the subsequent exposure of humans and the environment to these. Natural contaminants include naturally occurring radioactive material (NORM) and toxic trace metals. The MINEXRIM project, a collaboration between the Norwegian University of Life Sciences (NMBU), the Norwegian Radiation Protection Agency (NRPA), and the Norwegian Institute for Water Research (NIVA) under the auspices of the FRAM Centre (High North Research Centre for Climate and the Environment), aims to study the effects of potentially increased mineral extraction in the (Norwegian) High North made possible by the changing climate and concomitant increased accessibility to the high north. One necessary outcome of mineral extraction is the significant increase in surface area resulting from mechanical processes inherent in mining (e.g. crushing). Once the elements of interest are removed, the waste tailings are left with a modified and more importantly more available concentration of toxic and radioactive elements that are now no longer tightly bound in rock but more accessible to both aquatic (via leaching into the hydrosphere) and terrestrial (via e.g. earthworm feeding) ecosystems.

As their name suggests earthworms live in soil, but more critically for this work they also ingest large quantities of soil. Due to their ubiquitous nature, sheer quantity, and most importantly their diet, earthworms represent a significant vector for transfer of elements present in soil to terrestrial biota. Recent estimates suggest that there may be as many as 3000 unique earthworm species that in sum compose up to 80% of the total soil biomass (Lee 1985). Earthworm biomass in Europe has been estimated at 400-700 kg fresh weight ha^{-1}, and up to 2000 kg ha^{-1} in grassland areas (Edwards and Bohlen 1996). These worms are capable of ingesting and transporting soil significant distances both

vertically and horizontally in the subsurface (Müller-Lemans & van Dorp, 1996), and represent a critical vector for transfer of radionuclides and trace metals from soil to biota.

Understanding feeding patterns of earthworms is a prerequisite for studying uptake of key elements from soil to earthworms. Earthworms are capable of utilizing both organic matter tightly bound to mineral soil and more freely available subsurface organic matter present in soil as foodstuff, in addition to litter and animal feces above the soil surface. There is a strong species dependence for preference of soil type and soil vs organic matter, with some species favoring one soil type or one litter type over another and others displaying no preference at all (Curry & Schmidt 2007). Earthworms that feed deeper in the soil and rely primarily on soil organic matter are classified as *geophages,* and earthworms that reside higher in the subsurface and feed mainly on litter and other detritus are called *detritivores.* Some species have been reported to prefer mineral soils rich in organic matter over pure organic matter, and most species will feed on mineral soils when there is insufficient organic matter available (Doube et al. 1997). Earthworms can also be grouped according to which soil horizon the worms inhabit – *epigeic* worms inhabit the litter layer, *anecic* worms are deep burrowers and *endogeic* worms are shallow burrowers (Bouché 1981; Lee 1985). Uptake of nutrients is also possible directly through transport across the exterior surface membrane, although work done on Cd and Zn indicated negligible adsorption/uptake to the surface compared to uptake via ingestion (Vijver et al. 2005). It is reasonable to believe that other metals behave similarly, although this has not been shown conclusively.

Earthworms have been reported to consume in excess of 20-30% of their body weight in soil per day, varying due to nutrient availability in soil and litter (Brown & Bell 1995). Scheu (1987) reported daily consumption rates of 1.88-2.10 relative to the worm's biomass in *Aporrectodea caliginosa*, a detritivorous species. As geophages have relatively lower access to organic matter, they consume relatively more soil than do detritivores. Indeed, it seems that the earthworm feeding strategy is focused on high throughput of low quality material. At the extreme end, geophagous species living in

tropical soils with low organic matter content have been observed to ingest 6.7 times their body weight in soil per day (Lee 1985). Location, in addition to species, also seems to have a very important impact on ingestion rate, with ingestion rates of organic matter varying from 3 mg to 80 mg dry mass per day per gram fresh mass of worm (Curry & Schmidt 2007).

Toxic and radioactive elements are often evaluated through the lens of human and environmental health protection, and to this effect it becomes important not merely how much of these elements are present in which soils but how much is bioavailable and which mechanisms are present for transporting elements from their undisturbed (in soil/rock) state to a bioavailable state that may be of concern for plant, animal and human health. Mining and construction work, wood burning, weathering and biological activity are examples of anthropogenic and natural processes that can increase accessibility to nutrients as well as harmful elements.

Mining is often thought of as a local issue, and to an extent, this is true. However, on a global scale, cumulative land use by the mining industry was estimated to cover approximately 0.2% of the earth's surface at the end of the 20th century (Barney 1980). Environmental damage from mines and construction work is often related to mechanical damage of the landscape and acid mine drainage (AMD, Dudka & Adriano 1997). Mechanical landscape damage encompasses all form of mechanical activity that can result in reduced particle size/increased surface area of rock, which can lead to increased bioavailability. AMD can occur in areas where acid production - primarily due to oxidation of sulfides - occurs at a rate faster than it can be neutralized by alkaline materials in the waste, or where the buffering capacity is no longer sufficient (Salomons 1995). Much of Scandinavia is characterized by Fe- and S- containing rocks (Abreham 2007) that provide a source of sulfides for AMD. These deposits are often black shales or alum shales, and in addition to Fe and S may contain a wide spectrum of toxic metals and radionuclides (Statens Vegvesen Rapport 651, 2016). Surveys have been made on the effects of mining and construction in an alum shale-rich area on the local environment (Statens Vegvesen

Rapport 651, 2016), and understanding the role earthworms play in ecological transfer of toxic metals and radionuclides is of great ecological interest.

In nature, exposure to a single toxic compound is a rare occurrence, and in most cases there will be a multiple stressor scenario (Salbu et al. 2005). The experiment presented in this thesis represents such a scenario.

1.1 Objectives

By evaluating uptake of key radionuclides and trace metals in earthworms this thesis aims to present new data on both uptake of radionuclides and trace metals in earthworms in a laboratory setting and to compare laboratory data from this study to uptake data from earthworms collected in the field in this study and in other published works. The following hypotheses were tested:

1.1.1 Hypothesis 1

The U concentration factor by the earthworm *Eisenia hortensis* will be affected by increasing the concentration of trace elements while keeping the concentration of U constant due to multiple stressor effects. Null hypothesis: U concentration is unaffected by an increasing concentration of trace metals

1.1.2 Hypothesis 2

An increasing concentration of metals and NORM will impact earthworm health, as measured by physiological (body weight, survival) and reproductive (number of juveniles and cocoons) endpoints. Null hypothesis: an increasing concentration of metals and NORM will not affect earthworm health.

In addition, focus was placed on exploring the effects of metal/NORM soil concentrations on concentration ratios (concentration in worm relative to concentration in soil).

2 Materials and Methods

The study design was based on exposure of the earthworm *Eisenia hortensis* to 4 different types of soil with varying toxic metal and radionuclide concentration over a span of 70 days. The OECD Test no. 207 acute toxicity test guidelines (OECD 1984) were used as a basis for experimental design, substituting the use of artificially prepared soil with soil collected in the field. Toxic metal and U concentrations were measured in earthworms sampled from the four treatments after 1, 2, 4, 7, 10, 15, 22, 29, 36 and 70 days. In addition, the body weight of each sampled worm was recorded before and after depuration and at the end of the exposure period the total number of cocoons and juveniles was determined in each treatment. These factors were considered in the evaluation of the experimental hypotheses.

The field sampling was performed by Frøydis Meen Wærsted (NMBU) and Louise Kiel Jensen (NRPA) in August 2016, and the laboratory experiment was performed from October to December 2016.

2.1 Experimental design

2.1.1 Field work

The Orrefjell mountain region in northern Norway (68.89337 N, 18.10150 E) was selected as a sample site for work being done in the MINEXRIM project on the basis of known elevated natural U concentrations in the area (Rindstad 1981). Nine sites of interest were identified at the Orrefjell region,

and earthworms were found at four of the sampling stations (table 1 and figure 1). Stations 1, 5 and 8 are on pasture land, and station 7 is located at a site known to have an intrusion of U-bearing minerals.

Table 1: Orrefjell earthworm and soil sampling locations.

Sample site	**Latitude**	**Longitude**
Station 1	68.87733 N	18.07119 E
Station 5	68.87781 N	18.06855 E
Station 7	68.88366 N	18.10820 E
Station 8	68.87780 N	18.06816 E

Earthworms and soil samples were collected *in situ* and shipped to NMBU, where the earthworms were depurated, frozen, freeze-dried and digested prior to metal analysis via ICP-MS. Only worms from stations 5, 7 and 8 were analyzed for metal content since the worms from station 1 died prior to completion of depuration due to lack of moisture. In addition, the species of earthworm collected at each sampling location was determined by visual inspection by Emmanuel Lapied (NMBU) to be *Lumbricus Rubellus*. The soil was air-dried, sieved through a 2mm sieve to remove large particulate matter, and divided into subsamples for determination of pH, organic matter content, and analysis via ICP-MS.

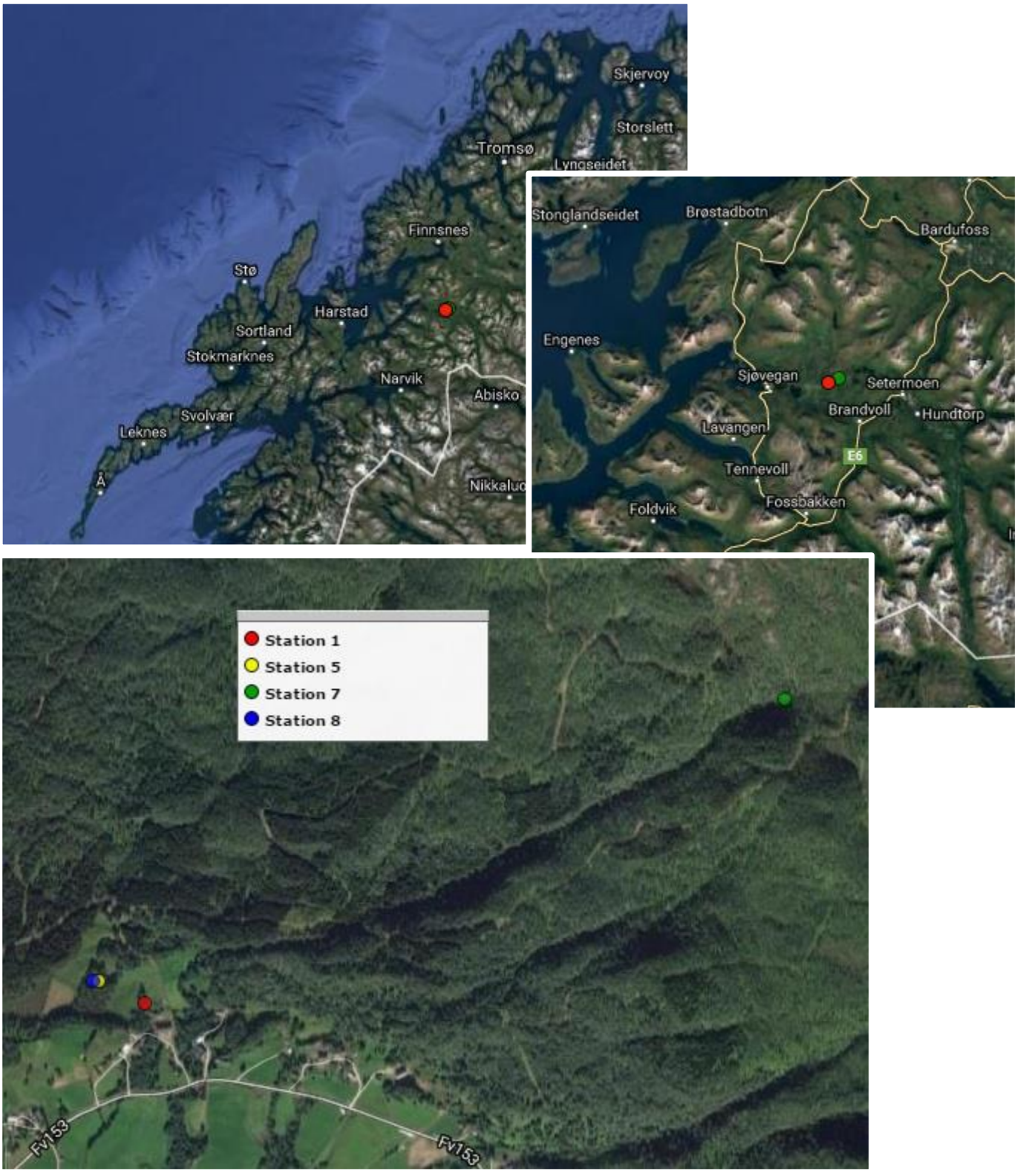

Figure 1: clockwise from top left: location of Orrefjell mountain in northern Norway, a close-up view of the sampling locations in relation to nearby towns, and a close-up view showing the position of all four sampling locations at the edge of a farming community.

2.1.2 Laboratory experiment

Soil

Soil was collected from Orrefjell station 7 in August 2016 and from Romeriksåsen (60.08066 N, 10.96901 E) in September 2016 and air-dried at room temperature over the span of five days to facilitate sieving and mixing with alum shale. Soil from Romeriksåsen (60.08066 N, 10.96901E) was chosen as a comparison soil due to the lower natural U concentration in the region (later measured to 80 Bq/kg vs 930 Bq/kg for Orrefjell station 7 soil). After the soil was dried, it was sieved to remove all particles greater than 2 mm. Crushed alum shale collected in 2015 from the tunnel construction at Gran (Statens Vegvesen Rapport 651 2016) was sieved and particles greater than 2mm were removed. Alum shale was chosen due to the similar U concentration relative to the Orrefjell St. 7 soil and the opportunity to observe the effects of multiple stressors in a relevant matrix (Salbu et al. 2005). Four treatments were prepared from these soils and the alum shale (table 2).

Table 2: The soil treatments prepared from dried, sieved soil.

Treatment	Base soil	Alum shale (w/w)
1	Romeriksåsen	0%
2	Orrefjell st. 7	0%
3	Orrefjell st. 7	5%
4	Orrefjell st. 7	25%

The treatments were each prepared in 2.75 kg batches and homogenized, and subsequently subdivided into five 550 g batches into sampling boxes for a total of five replicates for each of the four

treatments. A soil sample for ICP-MS analysis was taken from each soil prior to subdivision. The soils were then rewetted to mimic as closely as possible the condition of the soils when they were sampled (500 mL deionized water added to treatment 1, 300 mL to treatment 2, 275 mL to treatment 3 and 250 mL to treatment 4). At the conclusion of the experiment a soil sample was taken from each sample box for ICP-MS analysis. The time from alum shale addition and wetting to earthworm addition was one week.

The experimental boxes were transparent polypropylene plastic boxes (21 x 17 x 11 cm) with transparent lids, with the walls and bottom spray painted black on the outside to minimize light transmittance. 9 small holes (1-2mm) were punched in the lid to allow for gas exchange.

Soil pH was measured in water by taking 10 g dry soil and 10mL deionized water, mixing vigorously and allowing to settle for 15 minutes prior to determination of water pH by a pH meter, and % dry matter (105 °C) and %LOI (375 °C) were measured according to Konare et al., 2010. In addition, elemental analysis was performed on the soil to calculate total %C and %N using a Eurovector 3028 Elemental Analyzer (Carlo Erba).

Earthworm culture

250 g earthworms were purchased from a fishing supply company (Riverside Products, Mysen). The earthworms were purchased under the belief that they were the model species *Eisenia fetida*, but later analysis determined that they were *E. hortensis*. It was decided to proceed with *E. hortensis* due to the difficulty in sourcing sufficient numbers of *E. fetida* and the genus similarity between the two species. Furthermore, it has been noted that there is a need for more studies on species other than *E fetida* (Nahmani et al., 2007). The worms were delivered in a peat soil and left in this soil until 2 days prior to the start of the laboratory experiment and introduction to the treatment soils, whereupon they

were washed (deionized water) and placed in a batch of Romeriksåsen soil separate from the treatment 1 batch to be acclimated to pH and other soil conditions over 48 hours. 200 adult worms (wet weight > 400 mg) were then randomly assigned to the 20 experimental units (5 replicates of each soil with 10 worms per replicate), with worms that were visibly ill excluded. The worms were fed ground horse manure dried at 80 °C rewetted with deionized water weekly during the course of the experiment (0.5 ± 0.3 g/week). Dead worms, when present, were removed at each sampling point and at weekly intervals between the sampling events at day 36 and day 70.

Exposure conditions

The 20 boxes with soil and earthworms were kept on a table underneath a lamp set on a 16 hour on / 8 hours off diurnal cycle. The boxes were inspected regularly for water content and rewetted with deionized water. In addition, the box positions on the table were rotated every few days to minimize the effects of proximity to the light/heat source. The room temperature was maintained between 18 °C and 22 °C.

Earthworm sampling

One earthworm was sampled from each sample box after 1, 2, 4, 7, 10, 15, 22, 29, 36 and 70 days. The selected worms were rinsed in deionized water, weighed, and placed in separate small petri dishes (diameter 5.5 cm) on a wetted filter paper for depuration. After two days, the worms were again washed (deionized water), weighed and transferred to individual 15 mL plastic vials, where they were then euthanized by freezing at -20 °C. The bowel contents on the filter paper were left to air-dry for one

week, and then collected in individual sample boxes. After the experiment, the earthworms were freeze-dried to remove all water and subsequently digested and subjected to ICP-MS analysis.

Offspring

After the final sampling event, the soil in each sample box was inspected for cocoons and juvenile earthworms. The cocoons were collected from each sample box, counted and frozen. The juveniles were collected from each sample box, counted, and depurated (1 petri dish/sample box). During depuration some juveniles escaped their dish and some became mixed with juveniles from other sample boxes, making it impossible to be certain about the origin of all the juveniles. All the juveniles were frozen as a bulk sample, but no further analysis was performed on them. The cocoons from treatment 3 were pooled into three batches and analyzed via ICP-MS.

Mass spectrometric analysis

All elemental analysis was performed using an Agilent 8800 Triple Quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Sample preparation was performed by the author of this work, and analysis via ICP-MS was performed by Lene Valle at NMBU. The freeze-dried earthworms, along with the freeze-dried cocoons, were placed in 2.5 mL HNO3 and 1.0 mL H2O together with a Rh internal standard (4 μg/L in the final measured solution) in a Teflon vial. The biota samples were then submitted to microwave-assisted digestion in a Milestone UltraCLAVE, transferred to a 50 mL vial and diluted with mQ-water to 25 mL. The soil samples were analyzed by the same method with slight modifications. 0.2 g of the soil samples collected before and after earthworm exposure were placed in 2.5 mL HNO3 and 1.0 mL H2O, along with 1.0 mL HF, together with a Rh internal standard (4 μg/L in the

final measured solution) in a Teflon vial. The soil samples were then submitted to microwave-assisted digestion in a Milestone UltraCLAVE, transferred to a 50 mL vial and diluted with mQ-water to 25 mL. Visual inspection revealed incomplete digestion in every instance, with a small amount of clear crystalline precipitate remaining. It is suspected that some of the Si rock matrix remains intact after reacting with hydrofluoric acid, and that any trace elements bound within the non-decomposed sample is not bioavailable. The samples were then further diluted by a factor of 10 prior to ICP-MS analysis. NIST 2709a and NCS DC 73324 were used as certified reference materials for elemental concentration in soil, whereas IAEA 350 and NCS 7C 73014 were used for earthworms and cocoons.

Radiometric analysis

After the conclusion of the exposure period, the treatment soils were sent to the Norwegian Radiation Protection Authority (NRPA) for radiometric analysis of ^{226}Ra. The soils were dried at 105 °C for 24 hours prior to analysis. Radiometric analysis of ^{226}Ra was performed by assuming equilibrium with daughter products ^{214}Pb and ^{214}Bi and measuring both daughters via gamma spectrometry. The 352keV peak for ^{214}Pb and the 609 keV ^{214}Bi peak were measured and a weighted average taken, prior to back-calculation of the ^{226}Ra concentration in the soils (pers. comm. Louise Kiel Jensen/Østerås NRPA lab 14.03.2017).

3 Results and discussion

It is difficult to directly compare results from treatment 1 to treatments 2-4 due to the different base soil and the resulting difference in multiple variables (pH, % organic matter, water content,

elemental composition). Nonetheless, the treatment 1 soil provides an interesting reference as a soil with little U and generally low levels of toxic elements, and also provides an additional data point for the basis of comparing soil elemental concentrations and uptake in earthworms.

Throughout the discussion reference will be made to concentration ratios. A concentration ratio is simply the ratio of concentration of an element in earthworms divided by the concentration of an element in soil (IAEA 2010), as in equation 1:

Equation 1: calculation of concentration ratios

$$Concentration\ ratio\ (CR) = \frac{[\text{concentration X in earthworm}]}{[\text{concentration X in soil}]}$$

All concentration ratio calculations are based on the results of ICP-MS analysis on soils and earthworms presented in Appendix 1. Concentration ratios can also be referred to as bioaccumulation factors.

Concentration of metals analyzed via ICP-MS are determined by comparison of the signal strength of each element in a sample to a calibration curve (concentration vs signal strength), with analysis of known certified reference materials (CRM), ideally of the same type of sample matrix, as a quality control. Since the CRM's are prepared together with the samples, they will reflect both incomplete digestion and measurement errors. In the case of Mo, both CRM's used for earthworm analysis had values below near or below the limit of quantification due to high uncertainty in the Mo concentration in the analytical blanks. Mo concentrations for earthworms are reported in this work, but extra caution should be taken when considering these values!

All uncertainties presented in figures 2-21 in this section represent one standard deviation from the mean.

3.1 Soil analysis

The experimentally determined basic soil conditions are presented in table 3.

Table 3: Basic soil parameters. For pH, %C and %N n=5, for %DM and %LOI n=2.

	pH d=0 19 °C	pH d=70 22 °C	% DM	1σ	% LOI	1σ	%C	1σ	%N	1σ
Tr. 1	5.1	4.4	96.0	0.3	37.6	6.7	20.2	3.0	1.35	0.21
Tr. 2	5.3	4.5	97.2	0.6	23.0	2.5	11.1	3.7	0.98	0.27
Tr. 3	5.1	4.8	97.5	0.3	20.4	0.1	8.5	1.0	0.78	0.09
Tr. 4	5.6	5.4	97.9	0.2	16.1	0.2	8.6	1.7	0.73	0.20

It is interesting to note that the soil pH decreases over the course of the experiment, with the largest decrease observed in treatments 1 and 2 without alum shale. The alum shale added to treatments 3 and 4 was stored dry for one year prior to mixing with the soil and wetting one week prior to exposure, and it may be that it takes more than one week for the soil system to reach an equilibrium state. However, this would indicate greater changes in soil conditions in the latter two treatments, which is the opposite of what is observed. Sizmur & Hodson (2009) report in their review article both increased and decreased pH in soils after earthworm exposure, with a majority of studies showing increased pH as a result of earthworm activity. The direction and mechanism of this change is poorly understood and merits further inquiry.

Table 4 presents elemental concentrations in the treatment soils at day 70, the peat soil in which *Eisenia hortensis* was raised and delivered, and the horse manure fed to the worms. All soil measurements were taken both before earthworm exposure and after exposure at day 70. As only one sample of the peat soil and horse manure used to feed the worms were analyzed, it is not known how representative the reported values are of the whole.

Table 4: Results of ICP-MS soil analysis performed on soil sampled at day 70. The standard deviation shown is for the difference between replicate samples, where n = 5 for all treatments. For peat soil and dried horse manure only one analysis was performed, so no replicate standard deviation is available. The data is included as a reference. All concentrations are in kg dry weight.

Element	**Tr. 1** mg/kg	**1σ** mg/kg	**Tr. 2** mg/kg	**1σ** mg/kg	**Tr. 3** mg/kg	**1σ** mg/kg	**Tr. 4** mg/kg	**1σ** mg/kg	**Peat soil** mg/kg	**1σ** mg/kg	**Horse manure** mg/kg	**1σ** mg/kg
Mg	1800	200	7340	390	7060	390	6100	790	860	---	2700	---
S	2120	130	1120	110	3940	1700	10080	1900	1600	---	2100	---
K	10200	800	12000	700	13000	700	17600	1100	2100	---	15000	---
V	41	2.9	104	5.9	350	35	990	170	3	---	6	---
Cr	24	1.6	66	0.9	68	1.9	76	3.1	1.7	---	3.9	---
Mn	496	38	254	19	272	25	344	128	92	---	450	---
Co	5.7	0.3	6.4	0.4	8.0	0.3	11.6	1.1	0.6	---	1.0	---
Ni	12.2	0.4	19.6	1.5	57.8	5.2	170	14	1.3	---	4.2	---
Cu	13.6	1.1	33.0	1.6	42.4	1.8	72.4	13.6	14	---	25.0	---
Zn	88.4	3.3	65.8	1.8	120	7	296	22	46	---	110	---
As	2.7	0.3	1.6	0.4	8.7	0.8	26.8	5.3	1.0	---	0.2	---
Mo*	17.4	0.5	10.0	1.0	17.6	1.5	38.4	5.3	1.0	---	2.1	---
Cd	1.08	0.05	0.27	0.03	1.54	0.18	5.82	0.63	0.29	---	0.17	---
Sb	0.74	0.05	0.12	0.01	1.86	0.24	6.14	0.99	0.28	---	0.02	---
Pb	65.0	3.3	21.4	1.3	23.4	0.5	28.4	1.8	15.0	---	1.2	---
U	7.0	0.1	75.2	5.6	75.6	4.7	76.0	6.9	<0,2	---	0.3	---

Comparing the before- and after- soil analyses indicates that most elements display a slight but not statistically significant increase in concentration after exposure. However, two elements stand out in their behavior: Mg and K. Mg displays a 21-35% decrease after earthworm exposure, while K sees a 600% increase in treatment 1 and a lower but still significant increase in treatments 2-4 (155% increase in treatment 2, 113% increase in treatment 3, and 26% increase in treatment 4). Since the worms were coming from a relatively (compared to all treatments) Mg-deficient peat soil, it is possible that they were Mg-deficient and increased their Mg intake resulting in a soil Mg concentration decrease. The most likely explanation for the increase in K is the addition of K-rich horse manure throughout the course of the experiment, although it is questionable whether or not adding 5-10g horse manure to 550g soil can explain this increase entirely. The concentration of U in alum shale and in Orrefjell soil are indistinguishable, while a concentration gradient in S, V, Co, Ni, Cu, Zn, As, Mo, Cd, and Sb is demonstrated in treatments 2-4.

Based on the guidelines by the Norwegian Pollution Control Authority (NPCA, Statens forurensningstilsyn 2009), it is possible to rank the soil quality based on the concentrations of As, Pb, Cd, Cu, Zn, and Ni. In addition, the NPCA guidelines regulate soil quality based on Cr(III) and Cr(VI), but the speciation of Cr in this experiment is not known and thus if used must be compared to the lower of the two guideline values – Cr(VI). If all the Cr is assumed to be the more toxic Cr(VI), all four treatment soils would be classified as 'badly polluted [20-80 mg/kg Cr(VI)].' In the case of Cu, Zn, Cd and Pb all values fall within the categories 'very good' and 'good' quality. However, Ni and As concentrations exceed those for 'good' soil in treatment 4, necessitating the classification of soil in treatment 4 as moderately polluted (guideline for 'moderate:' As: 20-50 mg/kg, Ni: 135-200 mg/kg) in the absence of speciation information for Cr.

Radiometric analysis on the treatment soils (table 5) demonstrates elevated levels of ^{40}K and ^{226}Ra in treatments 2-4, and higher levels of ^{137}Cs in the Romeriksåsen soil.

Table 5: Results of radiometric soil analysis. The standard deviation shown is for the difference between replicate samples, where n = 5 for all treatments except for treatment 3 (^{40}K), where n = 4. All concentrations are in kg dry weight.

Element	Tr. 1	1σ	Tr. 2	1σ	Tr. 3	1σ	Tr. 4	1σ
	Bq/kg	Bq/kg	Bq/kg	Bq/kg	Bq/kg	Bq/kg	Bq/kg	Bq/kg
^{137}Cs	112.9	2.2	23.41	0.93	23.83	1.02	16.66	0.83
^{40}K	435.0	14.3	523.1	16.7	585.7	19.7	789.7	18.1
^{226}Ra	94	1.5	691	7	768	8	1024	11

In addition to the guidelines on soil quality, the Norwegian Ministry of Climate and Environment has guidelines for classification of radioactive waste (Klima- og miljødepartementet 2010), where the boundary between non-radioactive waste and radioactive waste is defined according to equation 2:

Equation 2: determination of radioactive waste category. C_k = specific activity for radionuclide k, $C_{e,k}$ = limit of specific activity for radionuclide k over which the waste is classified as radioactive

$$\sum \frac{[C_k]}{[C_{e,k}]} \geq 1 \rightarrow radioactive\ waste$$

Daughter products of natural uranium (e.g. ^{226}Ra) are not considered in determination of waste status in systems where natural uranium is present. Considering U (1 Bq/g U = 81 mg/kg U) in addition to ^{137}Cs and ^{40}K, equation 2 gives values of 0.33 for treatment 1 (not radioactive waste) and 0.99, 1.01 and 1.01 for treatments 2-4, respectively. Due to the high contributions from the specific activity of U and its daughters in these soils, they are all just on the border between not-radioactive and radioactive waste. Since there are almost certainly traces of other NORM nuclides in this soil that have not been considered here (e.g. ^{232}Th), these soils can be classified as radioactive waste.

^{137}Cs is present in lowest concentrations where alum shale has been added because alum shale has never been exposed to atmospheric deposition of ^{137}Cs. It appears that there may have been higher deposition of ^{137}Cs at the Romeriksåsen site vs the Orrefjell site as a result of the Chernobyl accident, but detailed ^{137}Cs deposition maps of these areas are not available.

^{226}Ra concentrations do not follow the U concentrations from table 4 in treatments 2-4 as more alum shale is added, likely because in the Orrefjell soil ^{226}Ra is removed from the soil by hydrological processes whereas it is still in secular equilibrium in the alum shale used in this experiment (crushed in the lab and not exposed to weathering). .

3.2 Elemental uptake / concentration ratios

In the following section, the uptake of elements in the earthworms is presented in the form of concentration ratios, and comparisons to other reported values are made. In all cases the number of sample points at each day per treatment is given by appendix 3. The day zero values are not treatment-specific but represent the concentration in 7 worms sampled prior to introduction of the worms to the treatment soils. These day 0 worms were transferred from the peat soil they were raised in to soil from Romeriksåsen for acclimatization alongside the treatment worms, but instead of being assigned to a treatment were instead depurated and frozen. The concentration ratio at day 0 is thus somewhat misleading since they were never in the treatment soil, but nonetheless serve to indicate the initial state of elemental concentration in the earthworms before exposure.

From the data presented in table 4 and appendix 1 it is possible to calculate concentration ratios for all elements presented in section 3.1 with the exception of the radionuclides presented in table 5, as data is available only for soil concentrations.

For the ease of visualization, concentration ratios are presented in subsequent figures for day 0, 1, 2, 15 and 70. Concentration ratios for all sampling days have been calculated and do not display significant deviations from the trends presented here. Where possible, relationships between soil concentration (day 70) and concentration ratios (day 70) are mathematically described.

3.2.1 Macronutrients - Mg, S and K

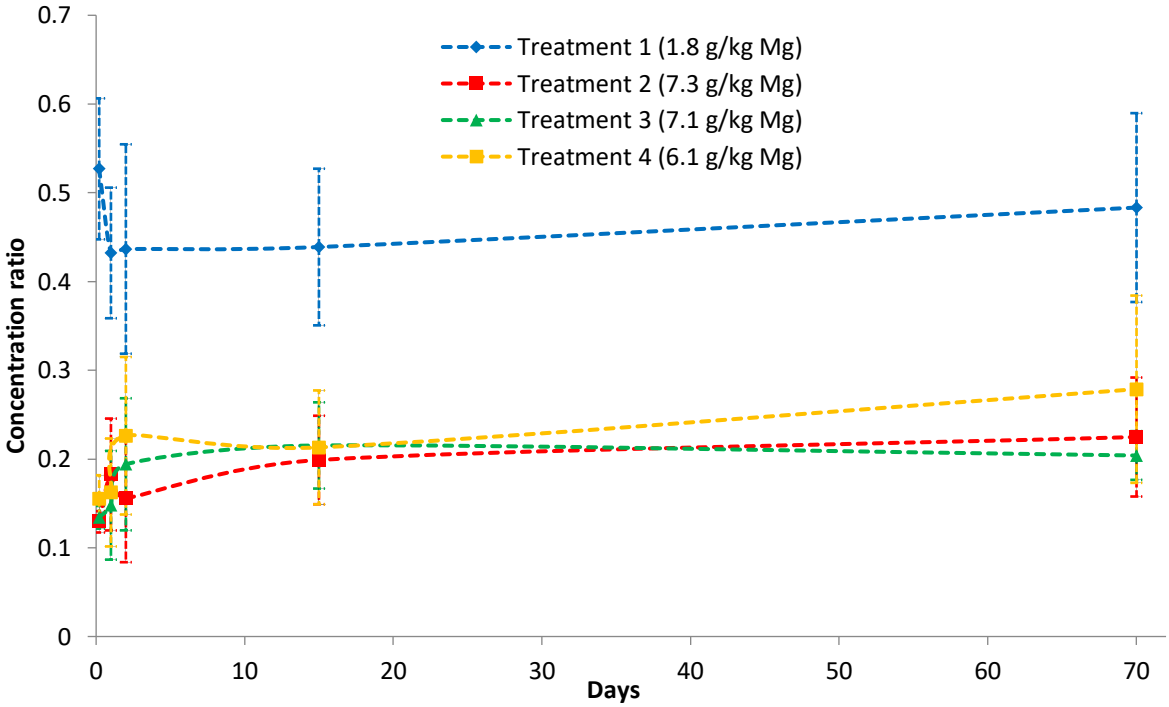

Figure 2: Concentration ratio of Mg in *E. hortensis* adult worms in four treatment soils.

After an initial adaptation phase, the concentration ratios for magnesium (Mg) in all four treatments stabilized rapidly and remained unchanged over the course of exposure (figure 2). The mean concentration ratio for treatment 1 with the lowest Mg concentration in soil (1.8 g/kg) is roughly double that of that in the other three treatments and the total Mg content in the treatment earthworms appears somewhat lower than in the other treatments (treatment 1: 0.87 ± 0.09 g/kg, treatment 2: 1.65 ± 0.40 g/kg, treatment 3 1.44 ± 0.11 g/kg, treatment 4: 1.70 ± 0.42 g/kg).

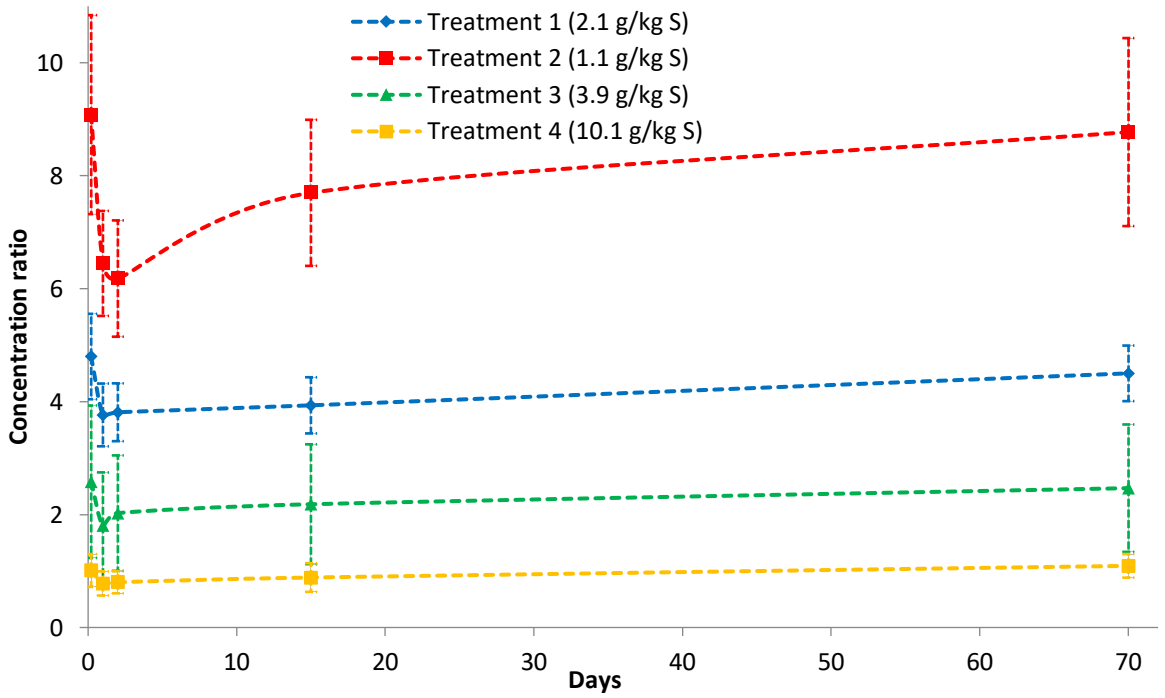

Figure 3: Concentration ratio of S in *E. hortensis* adult worms in four treatment soils.

Sulfur (S) concentrations (figure 3) also reach a stable level within each treatment after 15 days, and remain unchanged over the subsequent 55 days. With increasing soil S concentration there is a decrease in the associated concentration ratio, while the total S concentration is the same irrespective of soil concentration (treatment 1: 9.54 ± 0.46 g/kg, treatment 2: 9.83 ± 0.90 g/kg, treatment 3 9.72 ± 0.29 g/kg, treatment 4: 11 ± 1 g/kg). It is not surprising to see an increase in S after addition of alum shale, as alum shale is often S-rich (Statens Vegvesen Rapport 651 2016).

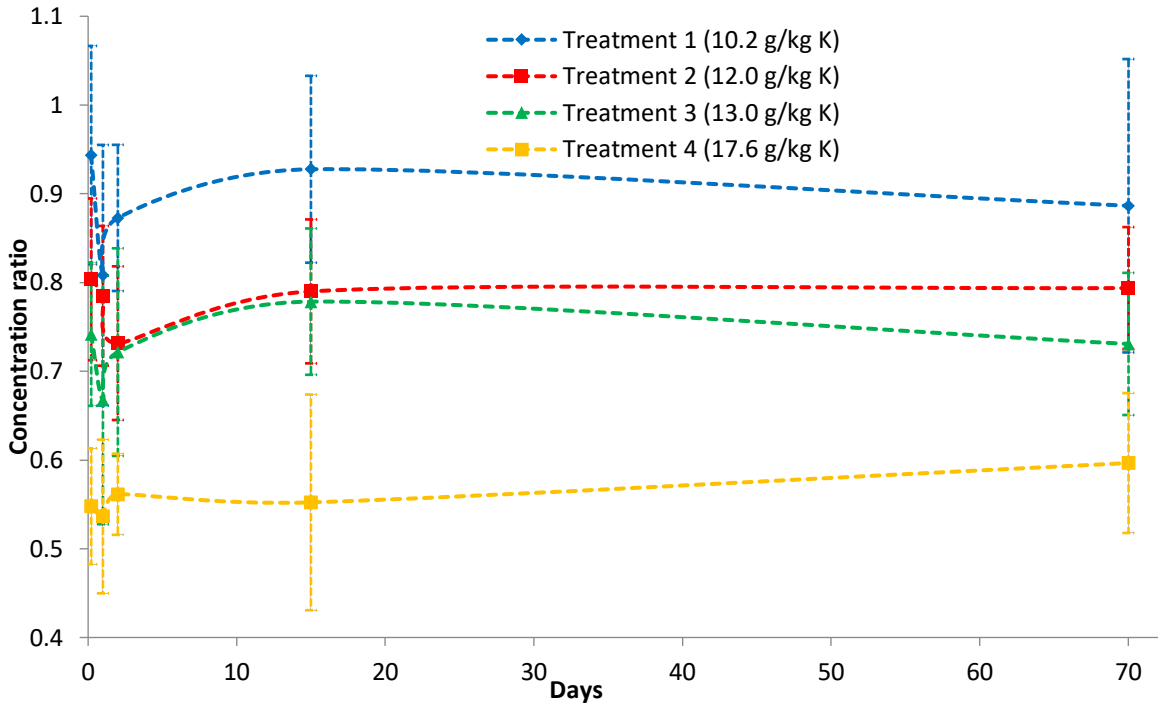

Figure 4: Concentration ratio of K in *E. hortensis* adult worms in four treatment soils.

As with S and Mg, a steady state is reached rapidly for the concentration ratio of potassium (K) in *E. hortensis* (figure 4). K displays a similar inverse relationship between soil concentration and concentration ratio to the other macronutrients, and it is not possible to distinguish the earthworms between treatments based on total K concentration (treatment 1: 9.06 ± 1.00 g/kg, treatment 2: 9.53 ± 0.26 g/kg, treatment 3 9.50 ± 0.52 g/kg, treatment 4: 10.50 ± 0.71 g/kg).

Closer investigation of the inverse relationship between soil concentration and concentration ratio in these macronutrients reveals a statistically significant inverse power relationship for all three elements based on day 70 steady-state concentration ratios (table 6).

Table 6: inverse power relationships between concentration ratio and soil concentration for Mg, S and K.

		Pearson's correlation coefficient (r)
Mg	$CR_{Mg} = 0.669\ [Mg_{soil}]^{-0.562}$	0.941
S	$CR_{S} = 9.253\ [S_{soil}]^{-0.939}$	0.999
K	$CR_{K} = 4.825\ [K_{soil}]^{-0.730}$	0.998

As these macronutrients play critical roles in everything from amino acid development to protein and peptide formation (Freney & Stevenson 1966, Morgan & Mitchell 1987), it is important to earthworms to be able to regulate these nutrients based on physiological need and not merely soil availability. This seems to be the case here – variations of an order of magnitude (S) and factors of 2-3 (K and Mg) are not reflected in total earthworm element concentration.

3.2.2 Micronutrients – Zn, Cu, Cr, Mn, Co, Mo, and V

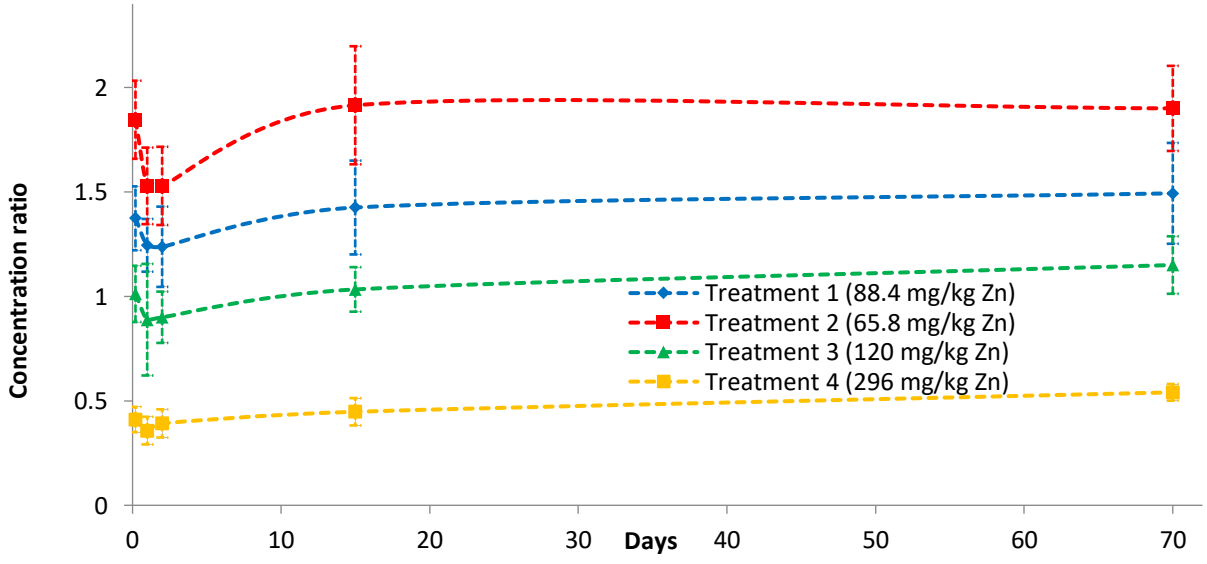

Figure 5: Concentration ratio of Zn in *E. hortensis* adult worms in four treatment soils.

Zinc **(**Zn) concentrations can be seen to stabilize at a steady state by day 15 (figure 5), with the concentration ratio showing a strong negative correlation to soil Zn concentration. This relationship can be mathematically described by equation 3:

Equation 3: the relationship between Zn concentration ratios and total soil Zn concentration

$$CR_{Zn} = 63.40\ [Zn_{soil}]^{-0.837} \qquad r=1.000$$

Concentration ratios have been reported for both higher and lower soil Zn concentrations than what is observed here. Van Hook (1974) observed ratios between 3 (30 mg/kg Zn in soil) and 13 (37-40 mg/kg Zn in soil) in *Alabophera, Lumbricus,* and *Octolasium* earthworms collected in Tennessee. Using equation 3 to estimate a concentration ratio from a soil Zn concentration of 30 mg/kg results in a concentration ratio of 3.7, which is in agreement with the reported values. In an extreme case, Zn concentrations in soil of 29000 mg/kg have led to reported concentration ratios of 0.064 in *Dendrobaena rubida* and 0.094 in *Lumbricus rubellus* (Morgan & Morris 1982), which are somewhat higher than 0.012 as predicted by extrapolation of equation 3.

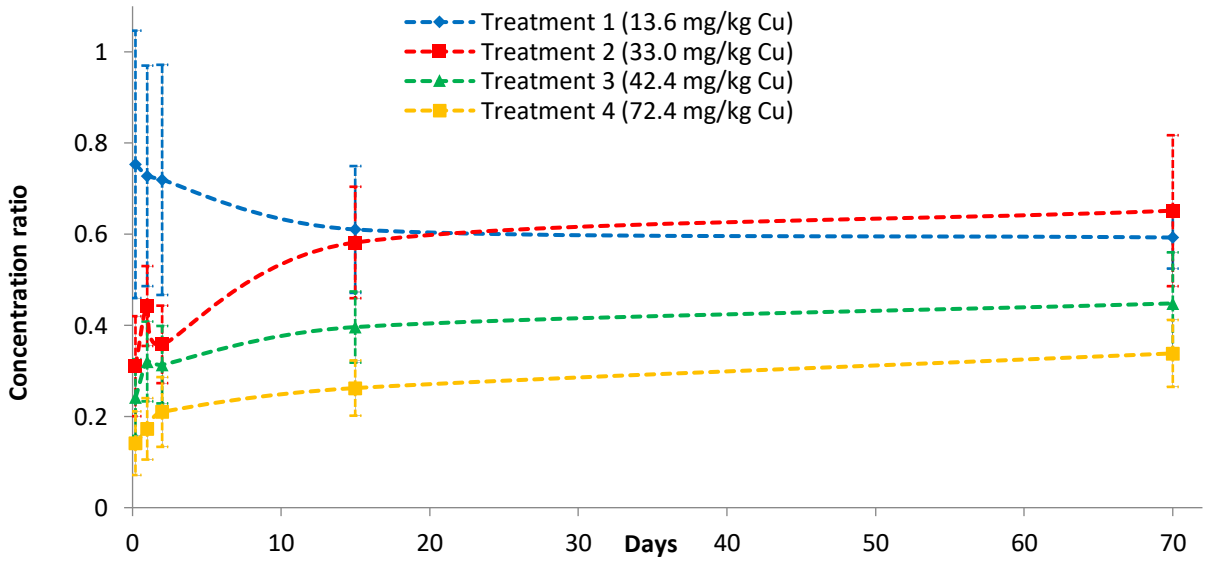

Figure 6: Concentration ratio of Cu in *E. hortensis* adult worms in four treatment soils.

There is a weak negative correlation between soil concentration of copper (Cu) and concentration ratio over the soil concentration range studied here (figure 6), but the data is insufficient to determine the form of the relationship. In all treatments the concentration ratios rapidly reach a stable level, and change very little between day 15 and day 70. This matches well with known data suggesting both Cu and Zn reach equilibrium concentrations in earthworms rapidly (Spurgeon 2010). Ma et al. (1983) report an average Cu concentration ratio of 0.54 (uncertainty unknown) near a smelting site in the Netherlands (soil Cu avg. 65 mg/kg), which is within the uncertainty range of the concentration ratios in all treatments presented in figure 6. Reported concentration ratios of 0.17-0.36 (*L. rubellus*) and 0.25.0.39 (*Aporrectodea caliginosa*) in soils containing 61-307 mg/kg Cu (Hobbelen et al. 2006) provide further evidence of a negative correlation between soil concentration and concentration ratio. The decreasing or flat trend for treatment 1 concentration ratios can be explained by the very similar Cu concentrations in the peat soil that *E. hortensis* was delivered in (14 mg/kg in one replicate) compared with the 13.6 ± 1.1 mg/kg Cu in the treatment 1 soil.

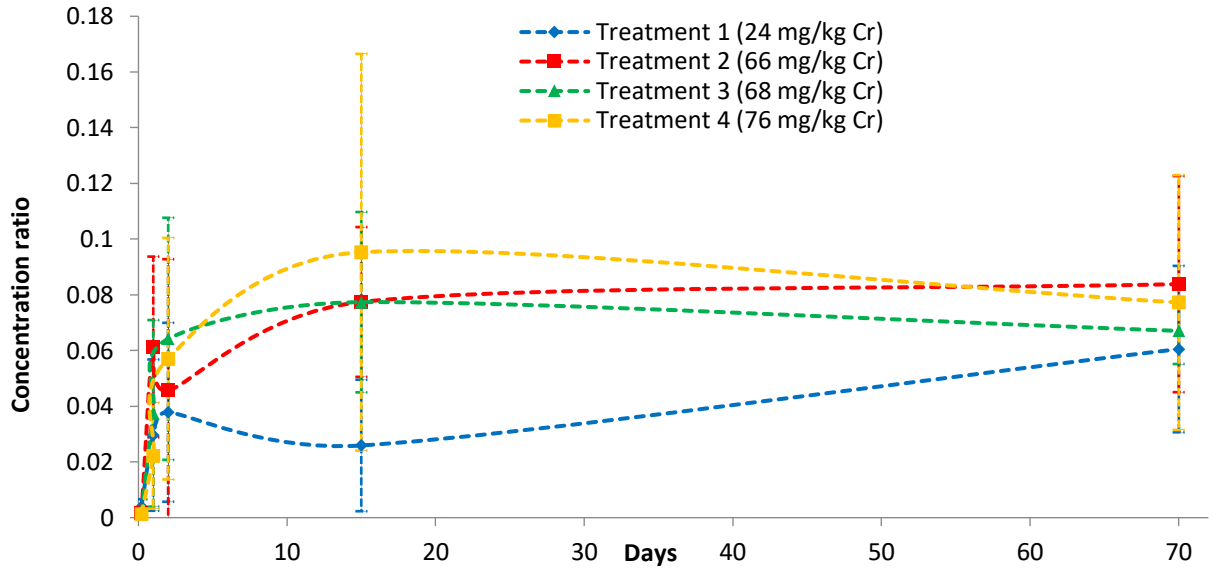

Figure 7: Concentration ratio of Cr in *E. hortensis* adult worms in four treatment soils.

Worms in all four treatments display statistically indistinguishable concentration ratios after 70 days of exposure to chromium (Cr, figure 7), despite there being 50-75% less Cr in treatment 1 soil relative to soil from treatments 2-4. While Cr (in particular, CrIII) is beneficial to living organisms in low concentrations (Zayed & Terry 2003), Cr(III) and Cr(VI) are both detrimental to organism health at higher concentrations. Cocoon production has been shown to be inhibited in *Eisenia fetida* (Lock & Janssen 2002) at 892 (679-1110) mg Cr/kg dry weight (EC_{50}), and lethal concentrations of 1656-1902 mg/kg Cr(III) in soil and 222-257 mg/kg Cr(VI) in soil have also been reported in *E. fetida* (Sivakumar & Subbhuraam 2005). The soil Cr levels (maximum 76mg/kg Cr in treatment 4) and earthworm Cr levels (maximum 5.90 ± 3.25 in treatment 4) in this study are all well below reported EC_{50} and LD_{50} values irrespective of oxidation state (not measured). The concentration ratio of 0.07 ± 0.03 is comparable to those obtained by Mrdakovic Popic et al. (2012) in *A. caliginosa* (0.12 ± 0.10) and *L. rubellus* (0.12 ± 0.07).

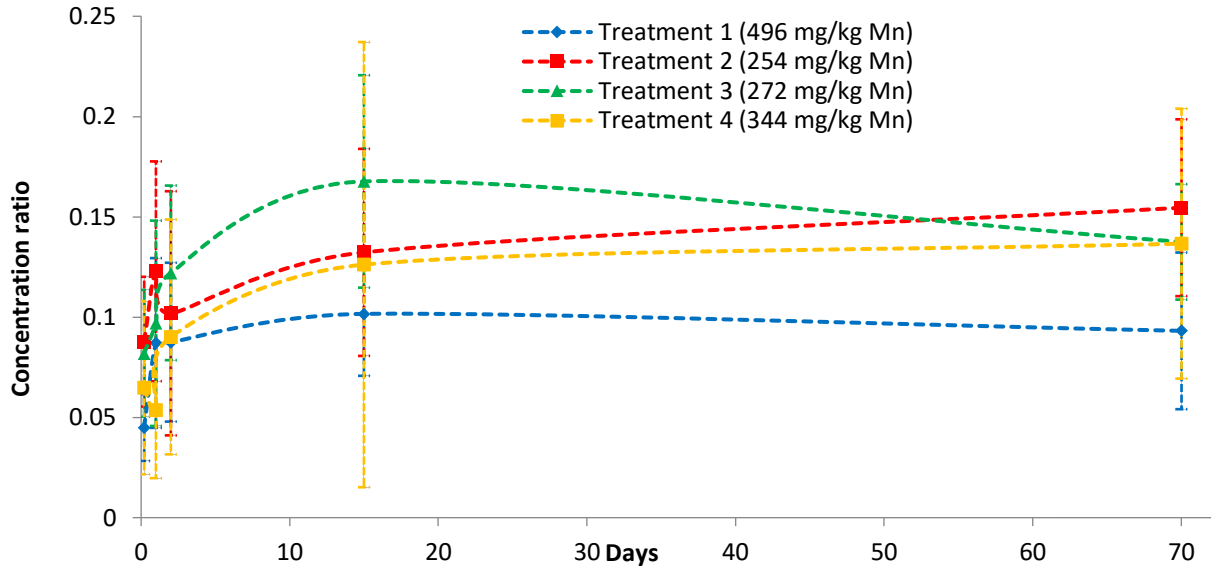

Figure 8: Concentration ratio of Mn in *E. hortensis* adult worms in four treatment soils.

Manganese (Mn) tends towards a negative correlation between concentration ratio and soil concentration (figure 8), but the uncertainty in concentration ratio measurements makes it impossible to draw statistically significant conclusions about the relationship between concentration ratio and soil Mn concentration. Soil concentrations of 3.7 g/kg Mn have shown concentration ratios of 0.19 after 36 days of exposure (Lourenço et al. 2011) in *Eisenia andrei*, while Morgan et al. (2007) report concentration ratios of between 0.010 and 0.100 for *L. rubellus* and *A. caliginosa* living in soils containing 9 to 41 g/kg Mn. The similarity of the values reported in literature to the values obtained in this work support the observation that there is no strong correlation between soil concentration and concentration ratio.

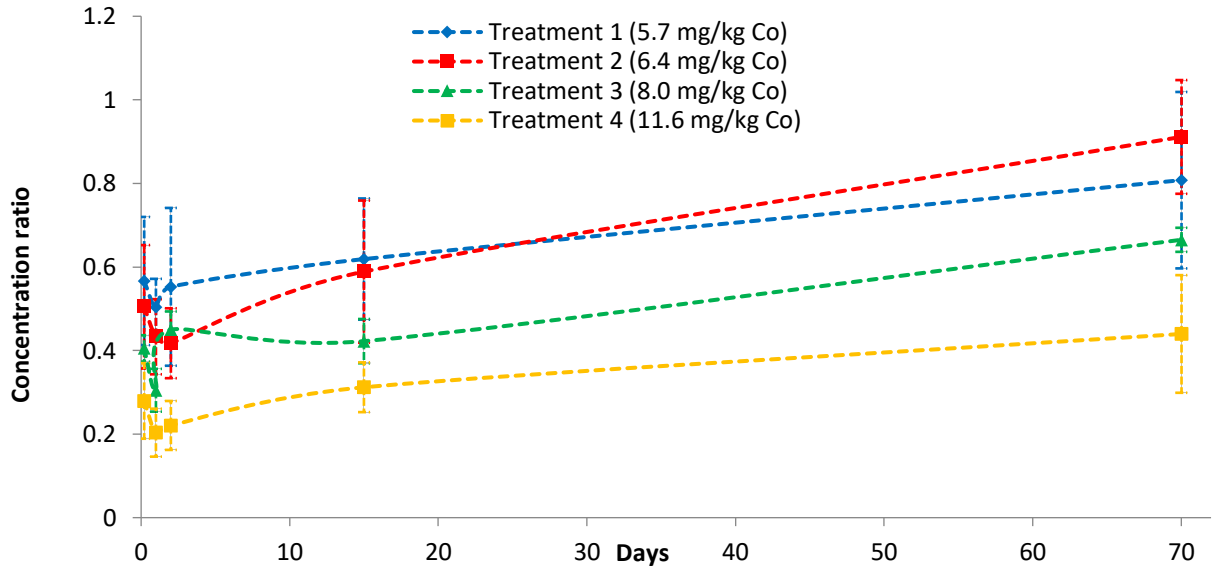

Figure 9: Concentration ratio of Co in *E. hortensis* adult worms in four treatment soils.

Cobalt (Co) concentration ratios (figure 9) appear to be increasing in all treatments throughout the duration of exposure (concentration ratios for days 22, 29 and 36, not plotted, follow the increasing trend visualized here), and they have not reached a steady state by day 70. The relationship between soil concentration and concentration ratio at day 70 can be described by equation 4:

Equation 4: the relationship between Co concentration ratios and total soil Co concentration

$$CR_{Co} = 4.9\ [Co_{soil}]^{-0.97} \qquad r=0.95$$

Rytkönen (2012) reports a median concentration ratio of 0.98 for pooled earthworms (*Aporrectodea* spp, *Aporrectodea Rosoa, L castancus* and *L. terrestris*) in soil containing 4.1 mg/kg Co in eastern Finland, which is comparable to the concentration ratios after 70 days for treatments 1 and 2 and with the concentration ratio of 1.24 predicted by equation 4 for a soil Co concentration of 4.1 mg/kg.

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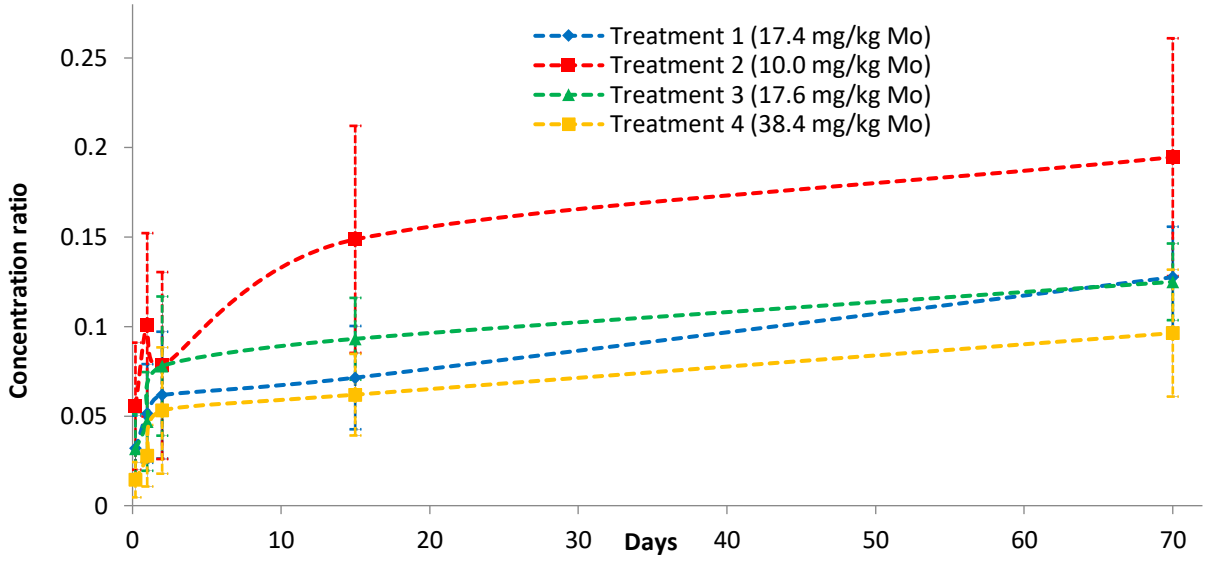

Figure 10: Concentration ratio of Mo in *E. hortensis* adult worms in four treatment soils.

Molybdenum (Mo) concentration ratios (figure 10) demonstrate a similar behavior to those of Co, with an increasing trend throughout the duration of exposure and an inverse relationship between concentration ratio and soil concentration that can be described by equation 5:

Equation 5: the relationship between Mo concentration ratios and total Mo concentration

$$CR_{Mo} = 0.575\ [Mo_{soil}]^{-0.51} \qquad r=0.96$$

Concentration ratios in soil from eastern Finland (Rytkönen 2016) match well with the predicted values based on this experiment (equation 5), with a reported concentration ratio of 0.58 in a soil with Mo concentration of 1.5 mg/kg near the predicted concentration ratio of 0.47.

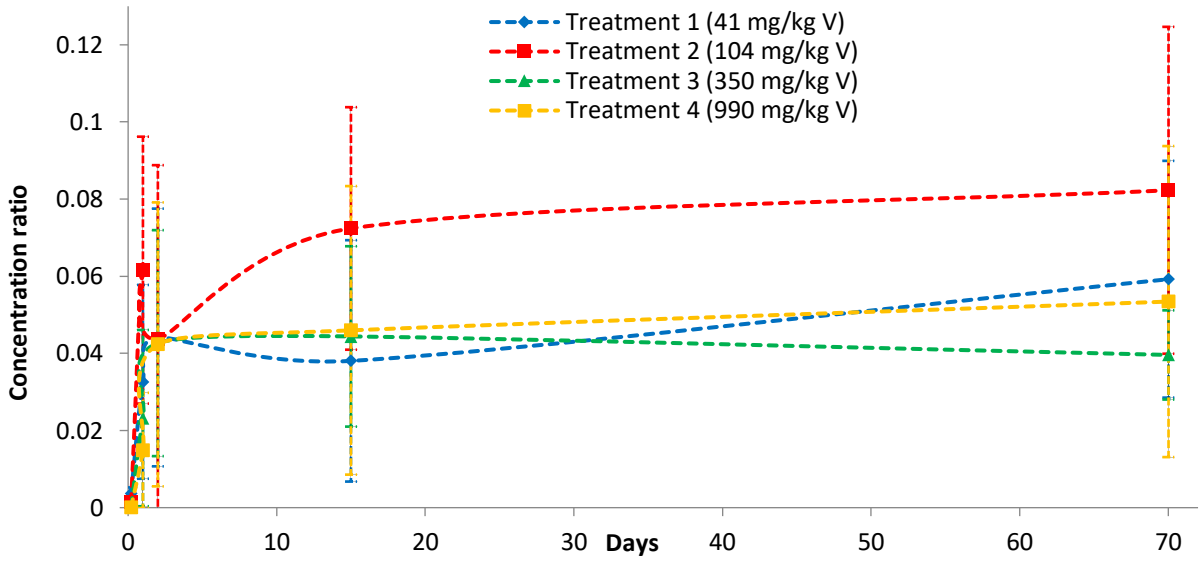

Figure 11: Concentration ratio of V in *E. hortensis* adult worms in four treatment soils.

Vanadium (V) concentration ratios (figure 11) seem to reach a stable concentration quite rapidly, although due to the low total V concentration there is a high relative uncertainty for these data. There is a tendency towards a negative correlation between soil concentration and concentration ratio as with all other elements, although there is insufficient data to say this with certainty ($p>0.05$). Very little V relative to soil concentration is taken up by *E. hortensis*.

3.2.3 Toxic metals – Ni, As, Cd, Sn, Pb

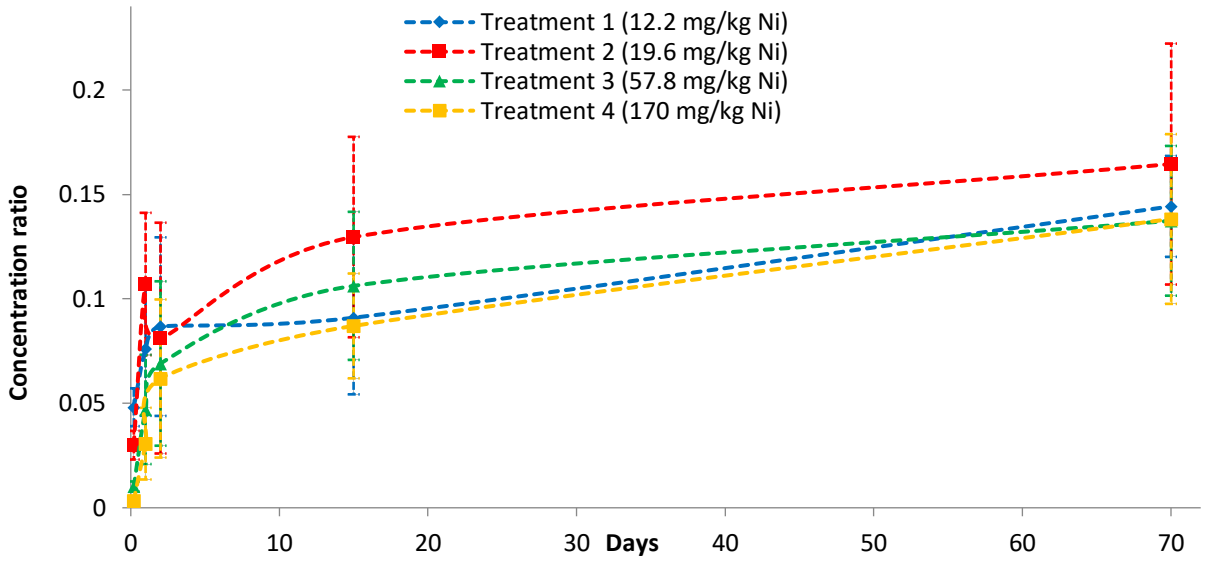

Figure 12: Concentration ratio of Ni in *E. hortensis* adult worms in four treatment soils.

There is no statistical difference in concentration ratios of Nickel (Ni, figure 12) between any of the treatments, despite the wide range of Ni concentrations in soil covered (12.2 mg/kg to 170 mg/kg). The concentration ratios appear to increase somewhat between days 15 and 70, but this effect, if present, is small and not statistically shown. Exposure to contaminated soil (91.4 mg/kg) for 56 days demonstrates a concentration ratio of 0.15 in *E. Andrei* (Lourenço et al. 2011), which is in agreement with the values after 70 days of exposure presented here.

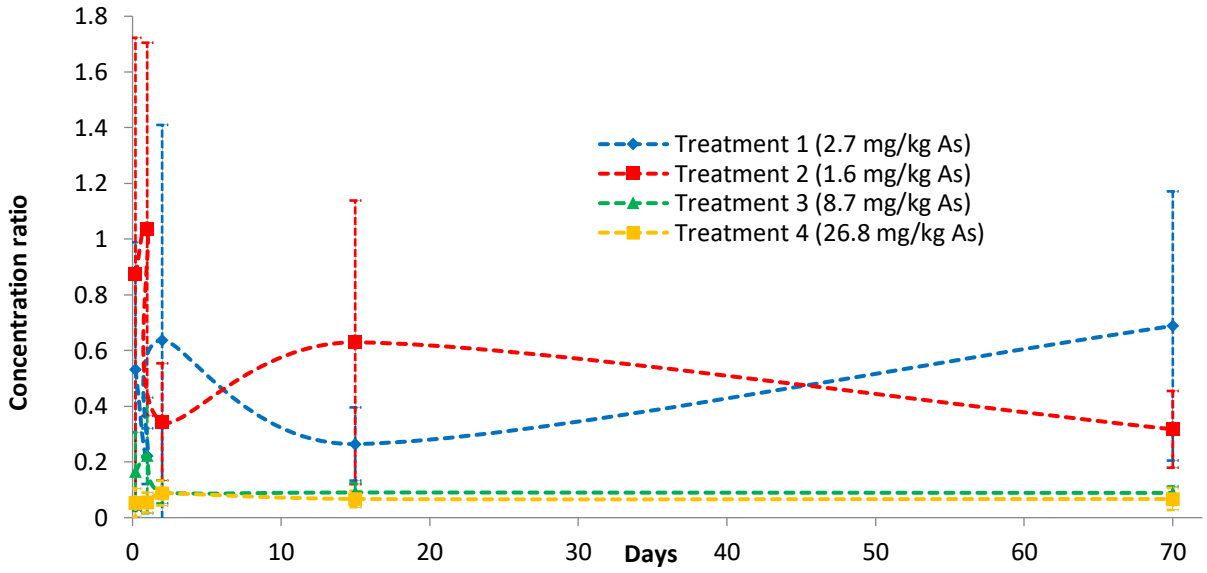

Figure 13: Concentration ratio of As in *E. hortensis* adult worms in four treatment soils.

Concentration ratios of arsenic (As, figure 13) compare favorably with the results reported by Mrdakovic Popic et al. (2012) for transfer factors of four different earthworm species sampled in situ in a high-NORM region of southern Norway (0.33 ± 0.25) in soils averaging 10-15 mg/kg As. There is a very high uncertainty related to As measurements so it is difficult to differentiate treatments 1 and 2, but a simple mathematical model of concentration ratio vs total soil concentration at day 70 does have some predictive power (r=0.86, equation 6):

Equation 6: the relationship between As concentration ratios and total As concentration

$$CR_{As} = 0.69\ [As_{soil}]^{-0.75} \qquad r=0.86$$

While this relationship is supported by Mrdakovic Popic et al. (2012), concentration ratios presented by Fischer and Koszorus (1992) show a much higher concentration ratio (10.3-18.1) in *E. fetida* for soils only moderately more contaminated (23-87 mg/kg As), while Geiszinger et al. (1998) report concentration ratios of 0.10-0.22 for soils containing 46-80 mg/kg As (*Lumbricidae)*. While there may be a correlation between concentration ratio and total soil As concentration, other soil factors appear have a stronger effect on earthworm As uptake.

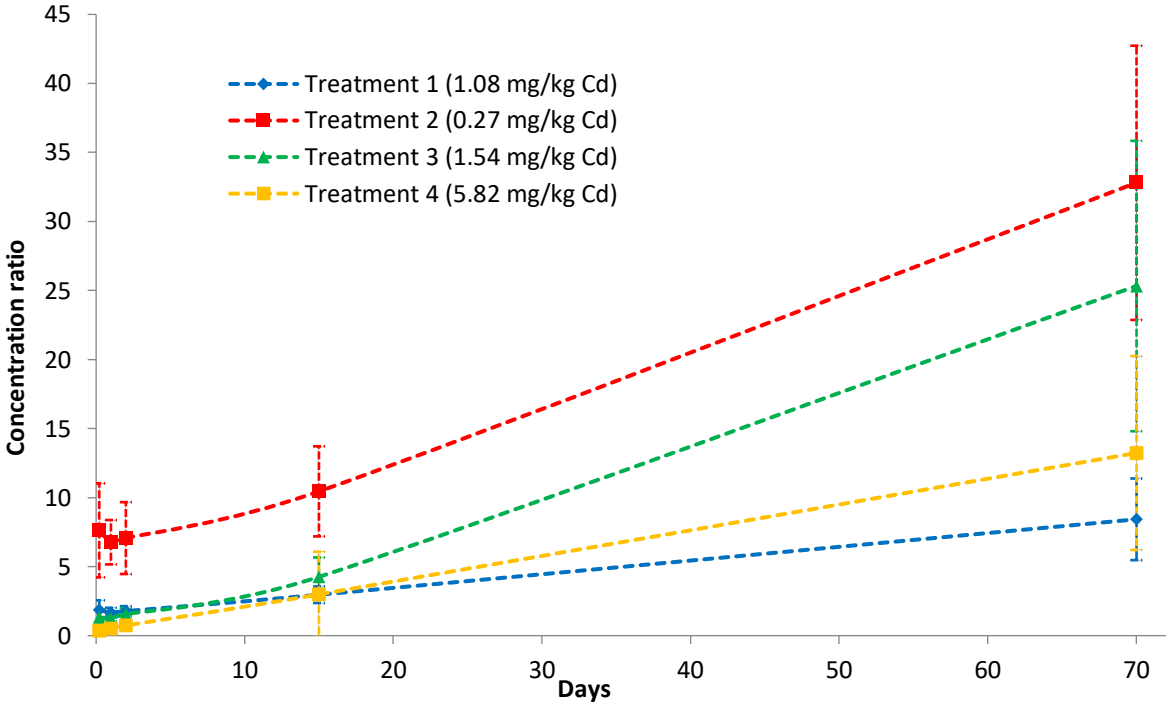

Figure 14: Concentration ratio of Cd in *E. hortensis* adult worms in four treatment soils.

As opposed to most other elements in this study, Cadmium (Cd) concentration ratios demonstrate an increasing tendency after 70 days in all treatments (figure 14). Cd, along with Pb, have been previously reported to have a long accumulation to equilibrium time (Spurgeon 2010). Cd exhibits concentration ratios well above unity, offering an explanation for why Cd is so toxic. It takes relatively very little Cd in soil to reach toxic levels compared to the other elements studied here. There appears to be a negative correlation between concentration ratio and soil Cd content, but the data is too uncertain to draw conclusions.

Mrdakovic Popic et al. (2012) report Cd concentration ratios of 10 ± 5 for a mixture of *A. caliginosa, A. rosea, D. rubidus,* and *L. rubellus* (species variation from 8-12 ± 3-5 does not indicate significant species difference) in soils with 0.2 to 1.9 mg/kg Cd in soil, which is comparable to the day 70 concentration ratios reported here (8.4 to 32.8 with the caveat that longer-term ratios may not be

comparable dependent on what the final equilibrium concentration ratios are – this requires further investigation). Van Hook (1974) reports concentration ratios of 11.6 to 22.5 in soils with 0.20 to 0.80 mg/kg Cd in soil, which also fall within the same order of magnitude as reported here. Data presented by Morgan and Morris (1982) indicate concentration ratios between 1.76 and 2.82 for highly contaminated soils (46 mg/kg Cd), supporting the observed tendency towards higher concentration ratios at lower soil concentrations.

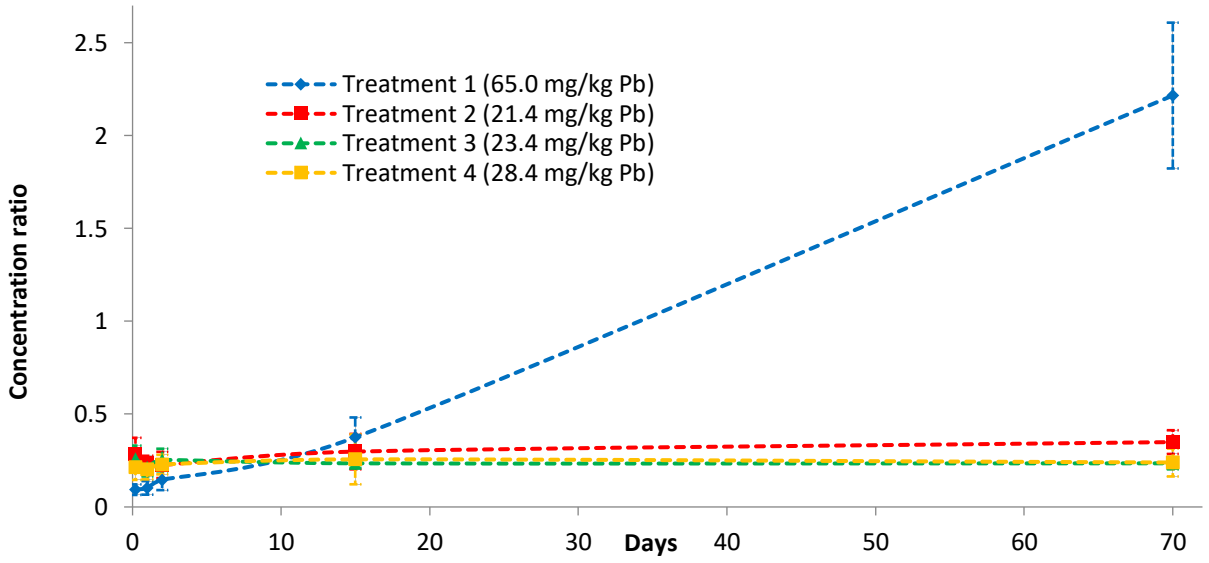

Figure 15: Concentration ratio of Pb in *E. hortensis* adult worms in four treatment soils. Pb is the only element measured that displays significantly higher concentration in treatment 1 worms relative to treatments 2-4.

Lead (Pb), as well as Cd, displays a slow accumulation to equilibrium in some treatments (figure 15). In treatments 2-4, it is possible that equilibrium concentrations are reached much more rapidly due to the similarity in Pb concentrations in treatments 2-4 (21.4 – 28.4 mg/kg) compared with the peat soil the worms previously resided in (15.0 mg/kg Pb).. The concentration ratios in Pb in treatments 2-4 compare favorably with the concentration ratios reported by Mrdakovic Popic et al. (2012) in earthworms found in soils with Pb concentrations between 16 and 159 mg/kg (0.14 ± 0.22), despite the

large uncertainty in the reported values. Van Hook's (1974) reported concentration ratios of 0.11-0.30 for soil concentrations from 15-50 mg/kg Pb follow the same trends as the concentration ratios from worms in treatments 2-4. In the highly contaminated soil (5486 ± 940 mg/kg Pb) reported by Morgan and Morris (1982), concentration ratios did not exceed 0.41 in *D. rubida* and 0.15 in *L. rubelluls*. This is confirmed by Lourenço et al. (2011), where a concentration ratio of 0.41 is reported after laboratory exposure to contaminated soil (9.7 ± 0.7 mg/kgPb) A survey of Canadian soils reported highly uncertain concentration ratios of 0.69 ± 0.89, 1.12 ± 2.05, and 0.74 ± 1.45 in a mixture of *Aporrectodea tuberculata* and *L. rubellus* at three different sites (Scheuhammer et al. 2003), which are on the order of magnitude of the treatment 1 concentration ratio (2.22 ± 0.39 at day 70 and possibly still increasing).

Low soil pH and low soil organic matter concentrations have been reported to lead to higher Pb uptake in earthworms (Ma et al. 1983). However, these effects are not seen here. There is no significant correlation between pH and concentration ratio in the worms studied, and there is a positive correlation between LOI% and concentration ratio after 70 days' exposure (can be expressed as a power function: $CR = 6.0e\text{-}5 * LOI\%^{2.84}$, r = .948). It is not known which factors are responsible for the differences in concentration ratio behavior reported in this work and that reported by Ma et al. (1983).

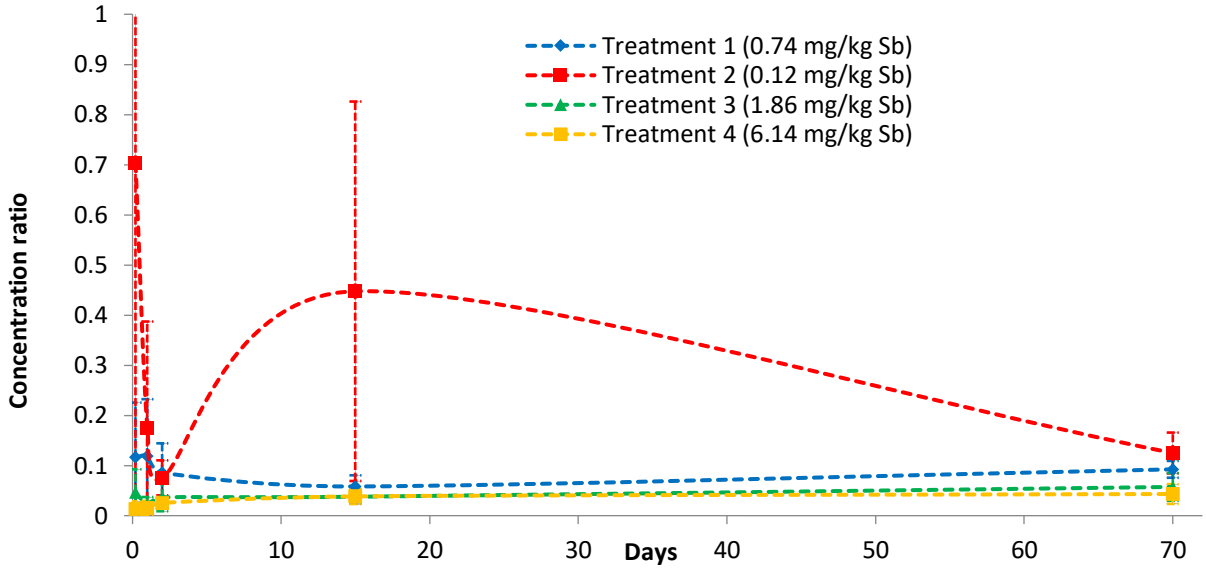

Figure 16: Concentration ratio of Sb in *E. hortensis* adult worms in four treatment soils.

Figure 16 visualizes the concentration ratio changes over time in the four treatment soils for antimony (Sb), with a large spike at day 15 in treatment 2. This elevated concentration ratio is also observable in the worms sampled at day 10 and day 29 but is not seen in any other elements, and it is unknown what the cause of this is. Day 36 (0.17) and day 70 (0.12) ratios appear to indicate that the concentration ratio has reached a stable level.

The very low concentration ratios after 70 days indicate very low transfer to biological systems of Sb from soil. This has also been observed in Leveque et al. (2013), with concentration ratios in *E. hortensis* reported between 0.004 and 0.11 in a soil concentration gradient of 0.05 to 210.5 mg/kg Sb.

3.2.4 Radionuclides/NORM – U

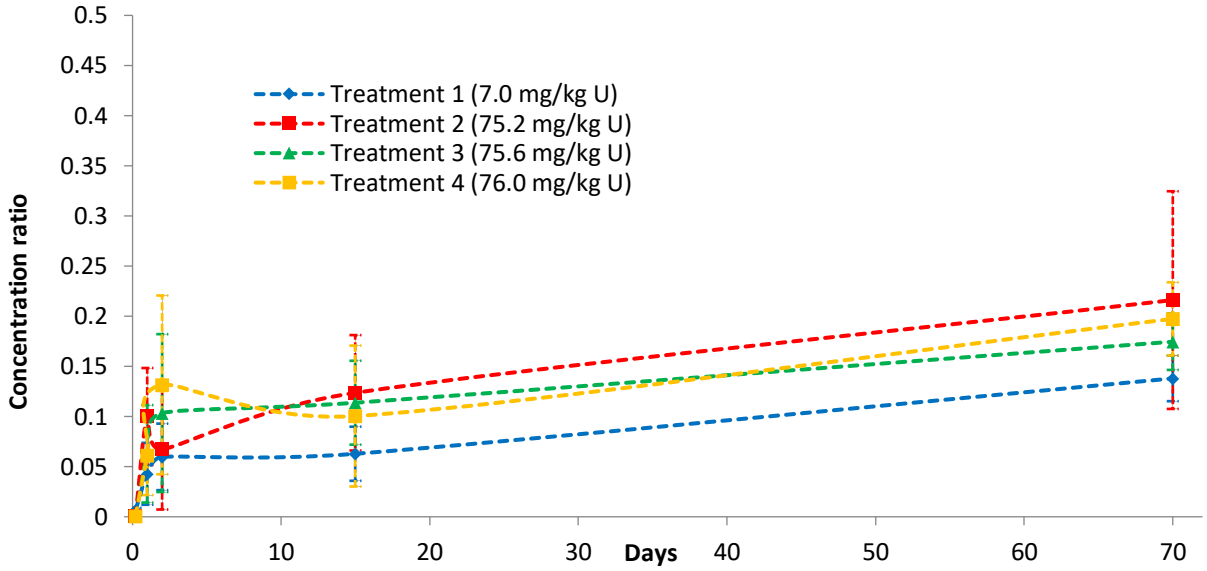

Figure 17: Concentration ratio of U in *E. hortensis* adult worms in four treatment soils.

Uranium (U) demonstrates rapid stabilization in concentration ratios in *E. hortensis* (figure 17), with a somewhat increasing concentration ratio over time reported in all treatments. There seems to be little variation between concentration ratios over the range tested (7.0 to 76.0 mg/kg U). The concentration ratios for U reported here (0.14 ± 0.02 – 0.22 ± 0.11) compare favorably with the results reported by Mrdakovic Popic et al. (2012) of 0.20 ± 0.16 in a soil concentration of 3-32 mg/kg U and with results reported by Lourenço et al. (2011) of a concentration ratio of 0.34 after 56 days in 215 mg/kg U soil. A study in low U concentration soils (Sheppard & Evenden 1992) presents concentration ratios of between 0.089 and 2.38 in soils containing "100 mg U/kg or less," with a correlation coefficient of -0.87 to cation exchange capacity and -0.80 to organic matter concentration. These correlations indicate that cation exchange capacity (CEC) and organic matter concentration (%OM) are likely responsible for most of the variation in reported concentration ratios, while pH displays a much weaker correlation (-0.12). The effects of CEC and %OM are not considered here.

3.2.5 Laboratory vs field-grown earthworms

In addition to comparing laboratory-obtained concentration ratios with published data, it is possible to compare these ratios with concentration ratios for the worms sampled in the field at Orrefjell. Concentration ratios for *L. rubellus* from stations 5, 7 and 8 are compared to those in *E. hortensis* exposed to the unadulterated Orrefjell station 7 soil (treatment 2) in figure 18.

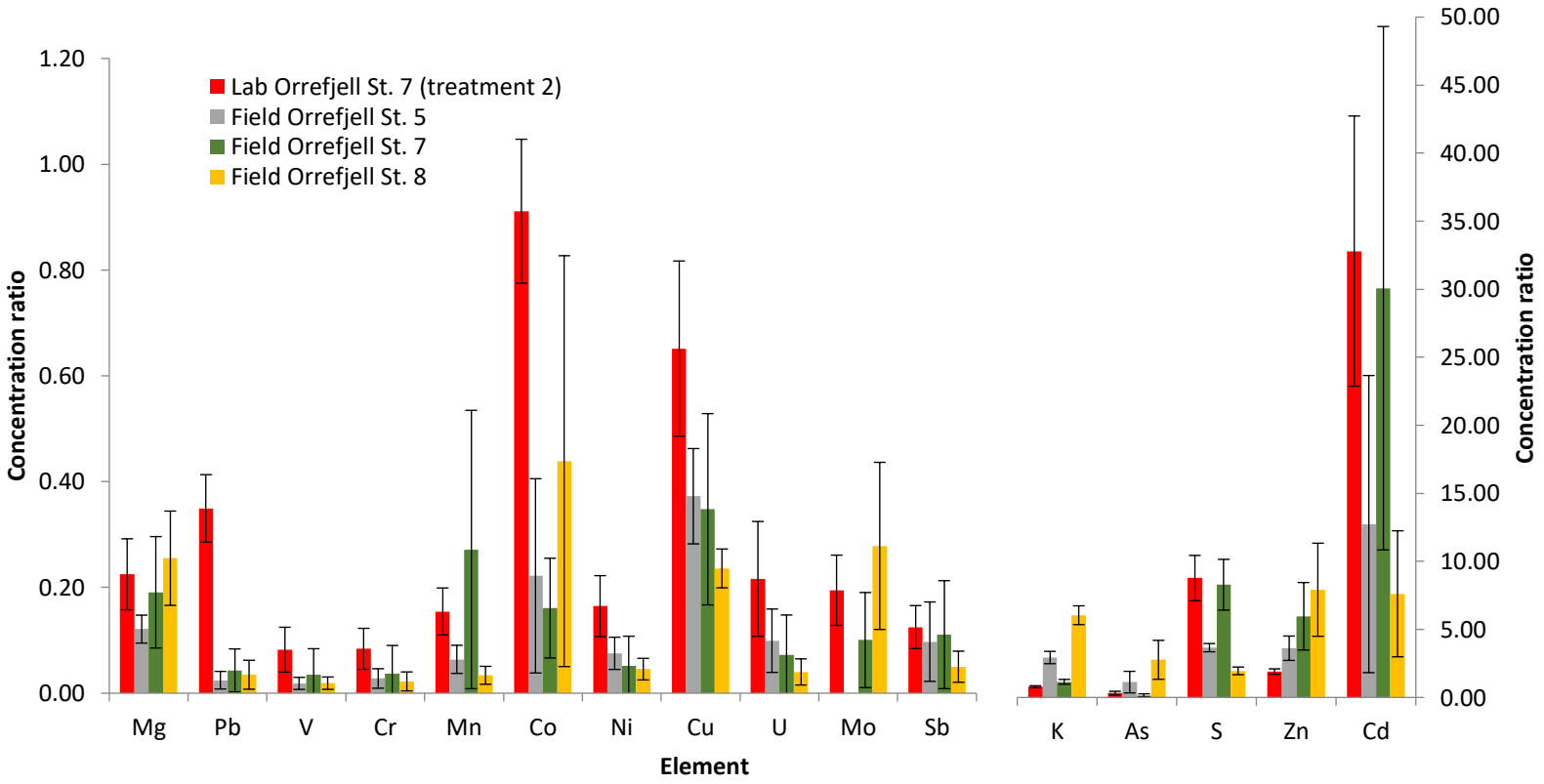

Figure 18: Concentration ratios for *E. hortensis* grown in a laboratory setting and *Lumbricus Rubellus* sampled at Orrefjell. All values for the laboratory exposure represent concentration ratios after 70 days of soil exposure.

Comparing the earthworms grown in the Orrefjell station 7 soil in the lab (treatment 2) with field sampled earthworms, one observes several elements that display significant ($p < 0.05$) variations in concentration ratios – K, Co, Pb, Zn, Cd and S. A reduced concentration factor in station 5 and 8 worms (respectively, 3.7 ± 0.3 and 2.0 ± 0.3) is observed with an increasing soil S concentration (respectively 2.9 ± 0.1 and 5.4 ± 0.1 g/kg) relative to station 7 concentration ratios (8.8 ± 1.7 lab, 8.3 ± 1.9 field ratios in soil containing 1.1 g/kg). This matches the predicted S concentration ratios at 2.9 and 5.4 g/kg S by the mathematical representation in table 6, section 3.2.1 (concentration ratio of 3.4 in 2.9 g/kg S soil and 1.9 in 5.4 g/kg S soil).

Potassium behaves similarly to S, with higher concentration ratios in station 5 and 8 worms and relatively lower soil concentrations (st. 5: concentration ratio 2.9 ± 0.5, soil concentration 3.9 ± 0.1 g/kg, st. 7: concentration ratio 6.0 ± 0.7, soil concentration 1.8 ± 0.1 g/kg) while the station 7 lab and field worms have indistinguishable concentration ratios. The equation for K concentration ratio in table 6 predicts the observed station 5 and 8 concentration ratios to within a factor of two, with a predicted ratio of 1.8 for station 5 and 3.1 for station 8.

There is a significantly lower uptake of Pb and Co in the field worms compared to laboratory worms. Furthermore, there is an observed higher concentration ratio in Zn in the field worms, and a lower cadmium concentration ratio in station 5 and 8 worms despite a similar soil concentration to station 7 (0.38 ± 0.03 and 0.32 ± 0.01 vs 0.27 ± 0.03 mg/kg Cd). It is possible these effects are a result of variation in concentration ratios across species for Pb and Co (*E. hortensis* in the lab vs *L. rubellus* in the field). This may be an effect of temperature (much lower in the field soils relative to the 20 ± 2 °C laboratory temperature) or earthworm mobility/field inhomogeneity, as in the field the worms are not limited to a small volume of soil and are free to travel to neighboring soils which may contain different soil Pb and Co concentrations. In addition, soil pH and % LOI are much higher for stations 5 and 8 versus station 7/treatment 2 (pH 6.0 for stations 5 and 8 vs pH 4.5 at station 7, LOI% 37% and 71% for stations

5 and 8 vs 23% for station 7, see appendix 4), which may explain lower concentration ratios at stations 5 and 8. It is also significant to note that stations 5 and 8 are farmland soils while station 7 is in a forest near a bedrock formation.

Despite the aforementioned differences, there was generally strong agreement between the station 7 laboratory worms and the station 7 field-sampled worms. This strengthens the case for the further use of laboratory studies in natural soils to complement field studies to further understand earthworms and their role in metal bioaccumulation and transfer. It is in many cases cheaper and/or easier to perform a study in the lab, and it is possible to tailor the soil to create element gradients of interest. Modified soils should, however, be allowed to reach an equilibrium between soil concentrations and soil water concentrations (also species equilibrium) prior to earthworm addition, to eliminate the effects of a possible confounding variable.

It is of great interest to perform further studies looking at the comparability of laboratory vs field-grown earthworms. This study is limited by sample size and a larger scale effort focused on reducing uncertainty may have implications in the use of laboratory-grown earthworms to study natural systems more effectively.

3.3 Physiological effects and reproduction

Physiological effects were measured in *E. hortensis* by using body weight at sampling as a proxy in addition to mortality. The wet weights of the earthworms measured immediately after depuration are visualized in figure 19.

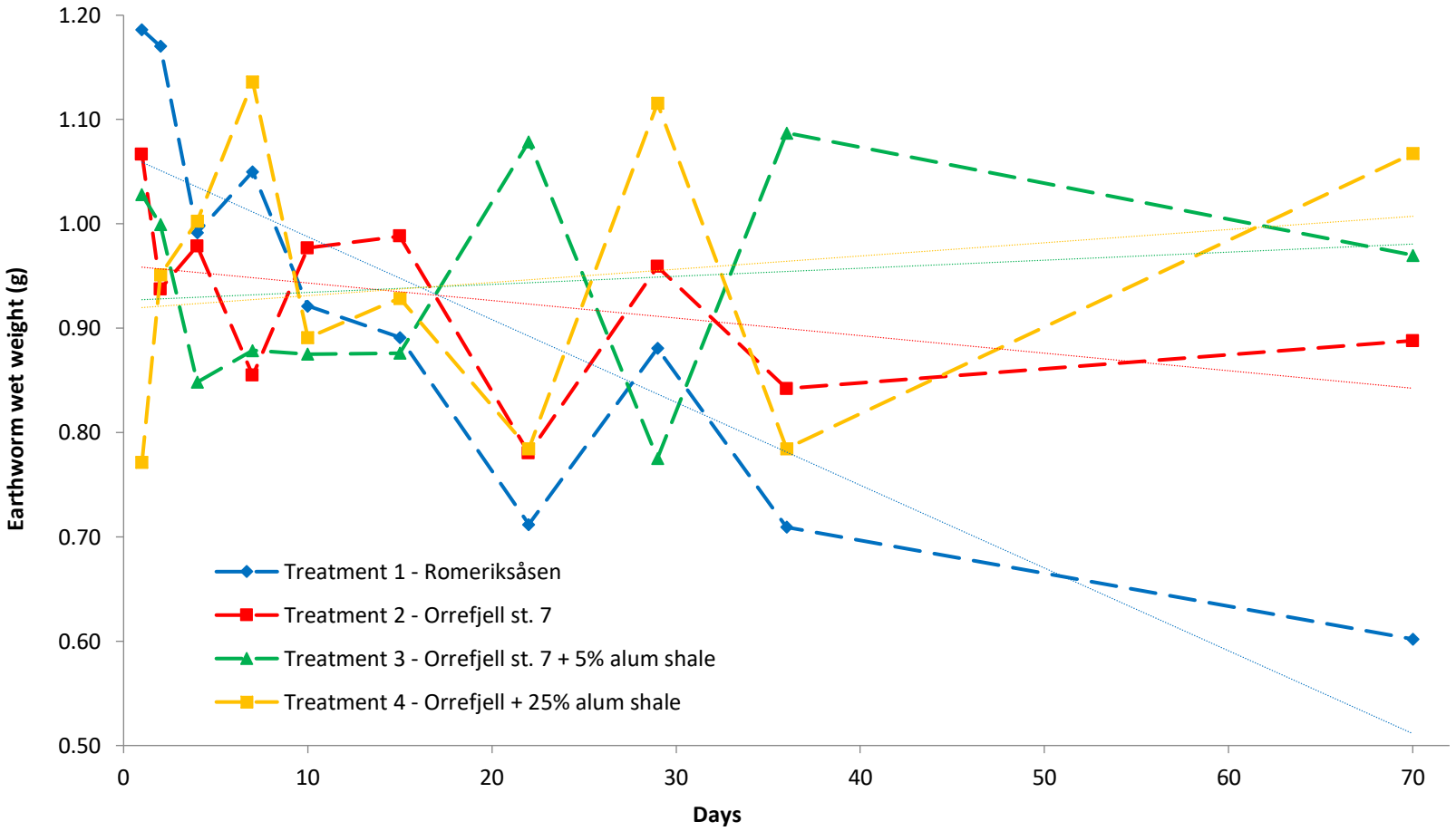

Figure 19: Average earthworm wet weight for each treatment at each sampling day. Error bars are not shown to improve clarity, but average between 35% and 40% for all treatments (see appendix 5 for full data set).

There appears to be a decreasing trend in earthworm wet weight in treatment 1 relative to treatments 2-4, with no significant differences between all three Orrefjell treatments. It is also possible to compare the treatments by looking at the mortality endpoint (figure 20). This shows a clear effect on treatment 4 worms, with a small effect in treatment 2 that may be due to random chance. Using wet weight as a proxy has limitations that become evident here as the natural variation in weight in adult worms necessitates larger sample sizes and/or more homogeneous starting conditions (e.g. select only worms that weigh 0.9 - 1.1 g for exposure instead of > 0.4 g as in this study).The full data set can be seen in appendix 3.

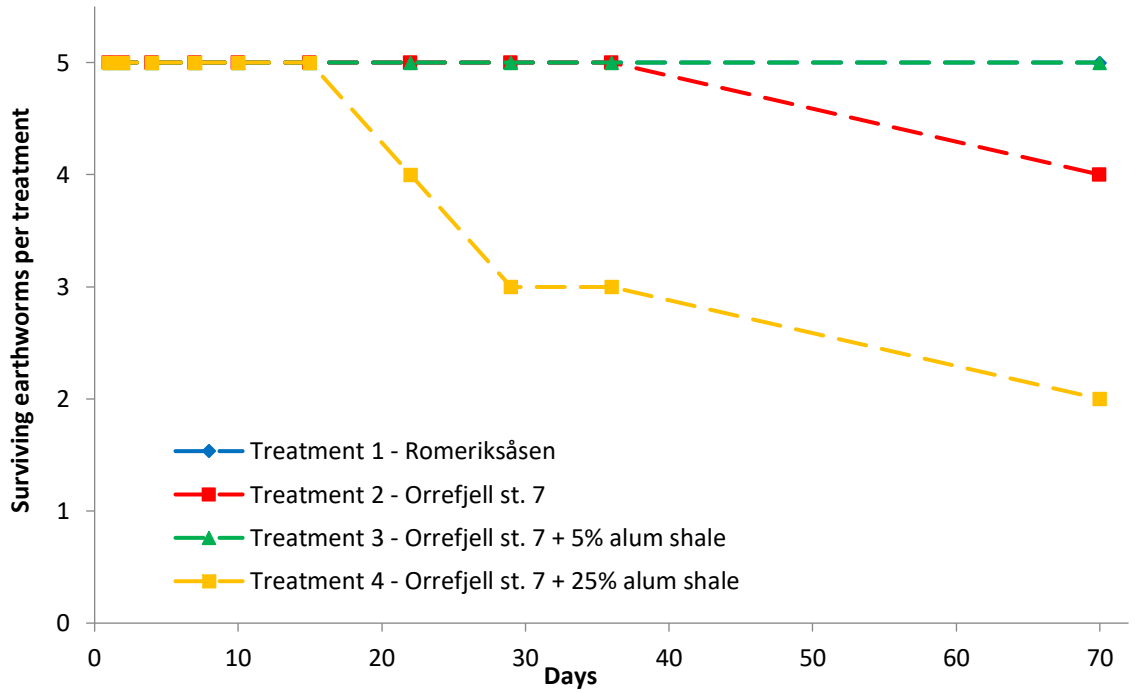

Figure 20: Earthworm survival per treatment. Treatments 1 and 3 are indistinguishable due to the survival of every worm in both treatments.

Based on the body weight and mortality endpoints, it seems that the earthworms in treatment 3 are the healthiest, together with the earthworms in treatment 2. Treatment 1 earthworms all survived

to the end of the experiment but exhibited decreased body weight, while treatment 4 worms showed normal body weight but experienced significantly greater mortality. It is possible that the treatment 4 worms that died showed decreased body weight prior to death, but this was not investigated further.

Table 7 presents the number of cocoons and juvenile earthworms found in each treatment at day 70. In addition to the cocoons, empty cocoon husks were found but these were not counted as it was in many cases difficult to distinguish them from soil and they were in most cases fragmented. They are also most likely already accounted for as juveniles.

Table 7: Observed cocoons and juveniles in the treatment soils

	Cocoons	**Juveniles**
Treatment 1	4	3
Treatment 2	19	17
Treatment 3	47	67
Treatment 4	8	11

Based on the results presented in table 7, treatments 2 and 3 appear to have favorable conditions for reproduction, with treatment 3 soil being preferred over treatment 2. It is possible that the addition of 5% alum shale contributes additional nutrients to the earthworms that are deficient in treatment 2 without affecting the organic matter content/pH significantly and without increasing total toxicity to a level that is damaging to reproductive health. However, earthworms in all replicates of 25% alum shale-enriched soil displayed lower reproductive rates than 0% and 5% alum shale-enriched soil, indicating one or more toxic elements reaches a level inhibiting earthworm reproduction. Spurgeon et al. (1994) report EC_{50} cocoon production (56 days) of 53.3 mg/kg Cu and 276 mg/kg Zn (estimated NOEC of 32.0 mg/kg Cu and 199 mg/kg Zn), both of which are exceeded by treatment 4 soil (72.4 mg/kg Cu and 296 mg/kg Zn). Earthworms in treatment 1 demonstrated worse reproductive health than those in treatments 2-4, and the combined body weight and reproductive data indicates that the Romeriksåsen

soil might not be ideal for *E hortensis* for reasons other than soil toxicity. This soil differs most from the other soils in % organic matter, although there is a moderate pH difference as well that may affect earthworm health.

It is interesting to note that increased earthworm mortality is observed in treatment 4 soil classified as 'moderately polluted' and 'radioactive waste' (Section 3.1), whereas earthworms in treatment 3 ('radioactive waste') and treatment 4, contrary to displaying negative reproductive effects, seem to increase reproductive activity. Current environmental standards (Statens forurensningstilsyn 2009) are focused on individual total element concentrations, and it may improve standard quality to consider multiple stressor effects as well as element concentrations (and speciation).

Cocoons

Concentration ratios have here previously been defined as concentration in an organism vs concentration in soil. However, as the cocoons cannot ingest soil and thus may receive their primary initial element load from their parent worm, it makes more sense to define a 'cocoon concentration ratio' as concentration in cocoon divided by concentration in parent worm. Cocoon concentration ratios between treatment 3 worms and treatment 3 cocoons are presented in figure 21.

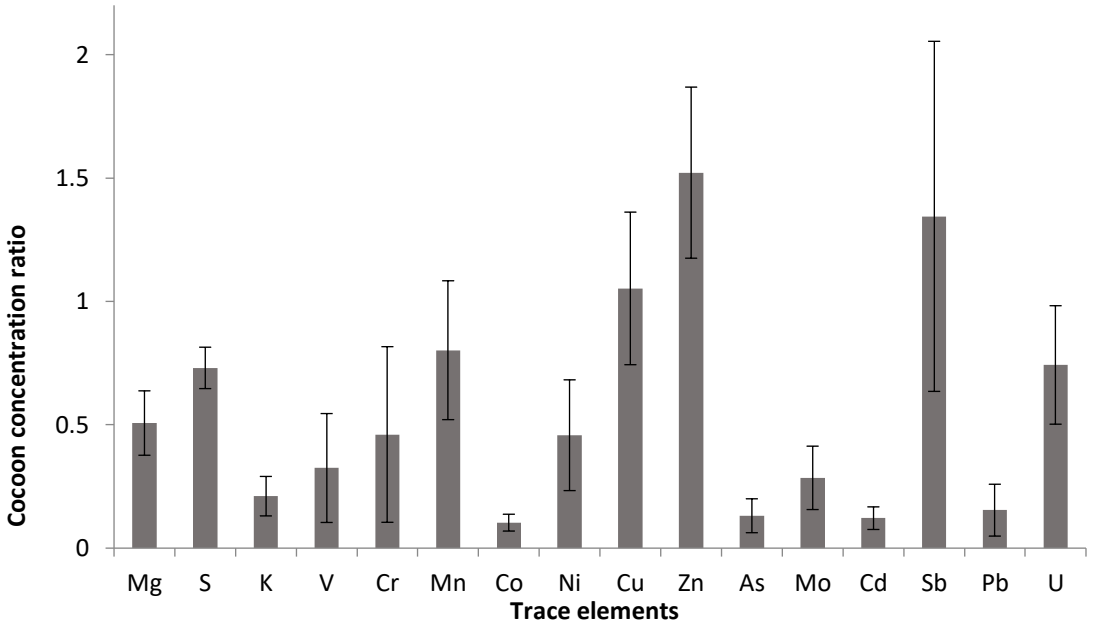

Figure 21: Cocoon concentration ratios for elements in *E. hortensis* cocoons relative to *E. hortensis* adults in treatment 3.

It can be seen that Zn and Sb are near or over unity, indicating an up-concentration of these elements in the cocoons. Cu also shows a ratio near unity, while Mn, U, and S are over 0.5. These elements all are passed on to the cocoons in significant concentrations, which may affect the development of the cocoons.

Many of the micronutrients discussed in section 3.2.2 have very low concentration ratios (Co, As, Mo, Cd), alongside Pb and K. In the conditions of this experiment, these elements have very little effect on bioaccumulation, and it is likely that these elements have little effect on cocoon health compared to elements with a higher concentration ratio.

4 Conclusions

4.1 Evaluation of hypotheses

Hypothesis 1

Based on the concentration ratios in section 3.2.4, there is little observed difference in uptake of uranium by *Eisenia hortensis* between any of the soil treatments. Thus, the null hypothesis that the concentration ratios for treatments 2, 3 and 4 are the same cannot be disproven and there is no shown effect in uranium concentration with an increasing gradient of other metals.

Hypothesis 2

The observed physiological and reproductive effects (section 3) indicate that an increasing concentration of metals and NORM does have a significant effect on physiological and biological endpoints. However, the sign of this effect is dependent on the metal/NORM concentration, as there is an increase in positive reproductive endpoints (number of cocoons/juveniles) with an addition of 5% alum shale. It is only with the addition of 25% alum shale and associated element load that mortality and reproductive effects start being observed. The null hypothesis that an increasing concentration of metals and NORM will not affect earthworm health based on the physiological and reproductive endpoints studied in this work is shown to be false.

It has also been shown that earthworms raised in laboratory conditions and in the field in the same soil have comparable concentration ratios for most elements (with the exception of Co and Pb, section 3.2.5), lending support to the use of laboratory studies as proxies for field studies where it is not advisable or prohibitively expensive to perform a field study.

4.2 Future work

All of the concentration factors in this work were calculated based on total soil concentrations. This has also been the case for most data reported on earthworm concentration ratios. However, it may be more ecologically relevant to discuss available concentration ratios, where the mobile soil fractions are considered instead of total soil concentration (Baeza & Guillen 2006). Performing a study similar to this one with a gradient of bioavailable element fractions of soil instead of a total element gradient and with an added focus on speciation (Salbu et al. 2004) would add greatly to the understanding of the earthworm's role in metal/NORM transfer and uptake.

Performing an uptake study over several generations may provide insight into how the earthworm system might be affected over time by e.g. a changing climate or the local influence of anthropomorphic activity, and to this effect it is interesting to evaluate transfer not only to cocoons but also to juvenile worms and track concentration ratios over several life cycles of an earthworm.

Excretion rates have been shown in several species to have a higher effect on equilibrium concentrations than the rate of metal assimilation (Spurgeon & Hopkin 1999). As the depurated gut contents of the worms in this experiment are available for study, a logical continuation of this study is to compare metal/NORM content in the gut content to concentrations in the earthworms and in the soil from this study. Additionally, a comparison of metal accumulation and excretion kinetics in earthworms exposed to contaminated field and laboratory soils may shed light on the relatability of laboratory experiments to field conditions.

Concentration ratios are often assumed to be constants for a certain earthworm species or certain type of soil. While it is known that this is an overly simplistic view, this study has shown that it is possible not only to relate soil concentrations to concentration ratios in many cases but also that these relationships can be used to predict with varying success concentration ratios in earthworms living in

other soil regimes. These simple relationships are based on a limited data set and focus on only the relationship between concentration ratio and soil concentration. Designing a model incorporating more parameters (soil pH, organic matter, soil type, cation exchange capacity and speciation) and based on a larger data set is an exciting (albeit ambitious) next step in understanding earthworms and their role in the terrestrial ecosystem.

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6 Appendices

6.1 Appendix 1 - ICP-MS analysis of earthworms and soil

Earthworms

LOD		5E-04	0,003	0,002	0,01	0,02	2E-04	0,002	0,02	0,2	0,1	0,002	0,2	8E-04	0,002	0,007	5E-04	0,001
LOQ		0,002	0,01	0,006	0,048	0,056	6E-04	0,007	0,056	0,72	0,38	0,006	0,51	0,003	0,006	0,023	0,002	0,004
		Mg	**S**	**K**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sb**	**Pb**	**Th**	**U**
		g/kg	g/kg	g/kg	mg/kg	mg/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Sample Name	comment																	
Blank 38	blank	<LOD	<LOD	<LOD	<LOD	<0,06	<LOD	<LOD	<LOD	<LOD	<0,4	<0,006	<LOD	<LOD	<LOD	<0,023	<LOD	<LOD
Blank 39	blank	<LOD	<0,01	<0,006	<LOD	<0,06	<LOD	<LOD	<LOD	<LOD	<0,4	<0,006	<LOD	<LOD	<LOD	<0,023	<LOD	<LOD
Blank 40	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	C1	0,99	9,8	9,9	0,13	0,092	0,024	2,8	0,54	9,6	110	1,4	<0,6	1,3	0,015	6,5	0,004	0,04
2	C2	0,98	12	8,5	0,15	<0,05	0,02	7,2	0,61	15	130	0,8	0,64	3,1	0,11	8,5	0,002	0,047
3	C3	0,98	9,4	10	0,25	0,13	0,021	2,3	0,78	8,5	120	2,7	<0,6	1,3	0,017	5,4	0,011	0,045
4	C4	0,95	9,9	9,7	0,17	0,14	0,016	4,1	0,55	13	130	0,86	0,5	2,3	0,087	5	0,006	0,074
5	C5	0,9	9,3	10	0,17	0,058	0,032	3,2	0,53	5,4	110	0,58	<0,5	1,7	0,17	4,3	0,003	0,023
6	C6	0,94	11	9,6	0,087	<0,07	0,029	3,8	0,56	11	120	3,2	<0,7	2,1	0,011	7,6	0,005	0,04
7	C7	0,9	9,8	9,8	0,095	0,06	0,014	6	0,54	9,2	130	0,49	0,53	2,4	0,19	5,1	0,003	0,052
8	4.1.1	1,3	7,9	11	29	3,2	0,025	3,1	7,7	14	100	2,4	1,6	3,6	0,17	6,8	0,3	8,4
9	4.1.2	1,1	7,5	9,7	18	1,9	0,025	2	5,5	13	100	2,6	1,2	3,7	0,11	5,5	0,14	4,8
10	4.1.4	2,1	7,6	11	99	10	0,056	3,3	22	19	120	2,4	4,6	7,5	0,27	7,1	1	20
11	4.1.7	1,3	9,3	12	47	4,2	0,028	2,9	13	16	120	1,7	2,3	15	0,25	5,5	0,3	7,9
12	4.1.10	1	9,5	10	7,1	0,67	0,026	4,1	7,2	16	120	0,53	1,2	11	0,11	3,3	0,054	5
13	4.1.15	1,3	9,9	12	27	2,9	0,023	4	13	19	140	1,1	1,7	42	0,19	6,2	0,22	7,2
14	4.1.22	1,1	10	11	15	1,5	0,028	5,5	10	19	120	0,87	1,4	46	0,11	8,5	0,091	11
15	4.1.29	2	9,3	11	69	9,1	0,059	3,5	24	24	150	2,2	3,9	32	0,29	6,8	0,83	20
16	4.1.36	1,4	11	12	22	2,6	0,032	3,5	13	21	150	0,89	2,4	62	0,17	5	0,2	8
17	St. 7 # 1	1,1	8,9	15	0,28	0,17	0,023	0,43	0,17	6,9	490	0,21	<1,1	4,5	<LOD	0,11	0,008	1,6
18	St. 7 # 2	1,2	9,5	13	2,1	1,3	0,043	1,2	0,86	13	230	0,18	0,72	8,7	<0,008	0,58	0,1	5,3
19	St. 7 # 3	1,9	9,1	13	8	5,7	0,13	1,5	2	13	370	0,36	1,3	7,8	0,016	1,6	0,38	6
20	St. 7 # 4	1,4	9,6	14	3,9	2,5	0,08	1	0,99	13	480	0,25	1	11	0,011	1,4	0,22	8,8

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
21	St. 5 # 1	0,93	11	11	0,49	0,26	0,014	2,3	1,3	22	250	2,9	0,99	4,1	0,008	0,11	0,015	0,45
22	St. 5 # 2	1,3	9,8	13	1,9	1,3	0,034	1,7	2,4	19	280	0,44	0,76	3,2	0,017	0,44	0,15	0,88
23	St. 5 # 3	1,1	11	10	2,3	1,5	0,028	6,1	3,4	23	400	4,8	1	11	0,032	0,67	0,19	1,6
24	St. 5 # 4	1,3	11	13	1,2	0,79	0,028	1,3	2	22	260	0,43	<1,2	2	<0,015	0,3	0,094	0,78
25	St. 5 # 5	1,2	9,5	12	2,6	1,9	0,029	1,5	2,8	13	260	2,5	<0,6	0,99	0,025	0,66	0,23	1,3
26	St. 5 # 6	1,1	11	12	0,56	0,31	0,015	0,83	1,1	20	250	2	<0,9	3,4	<0,012	0,071	0,011	0,31
27	St. 5 # 7	0,96	11	9,1	1,2	0,74	0,022	1,8	2,3	27	250	2,7	1,1	9,2	0,015	0,33	0,092	0,93
28	St. 8 # 1	0,99	11	11	0,75	0,34	0,016	0,89	1,1	10	150	1,6	<1,6	1,5	<0,02	0,38	0,035	0,74
29	St. 8 # 2	0,87	11	10	0,31	0,082	0,012	1,2	0,55	9,4	220	1,5	0,92	2	<0,005	0,12	0,001	0,35
30	St. 8 # 3	0,99	12	11	0,8	0,41	0,02	2,9	1,3	9,6	340	2,4	1,1	4,9	0,012	0,51	0,047	1,2
31	St. 8 # 4	0,81	9,6	11	0,25	<0,07	0,012	0,91	0,42	9,6	210	1,7	0,7	1,5	<0,008	0,13	<LOD	0,28
32	St. 8 # 5	0,88	12	10	1	0,38	0,028	5	1,5	9,7	140	3,9	1,7	4,6	0,012	0,89	0,037	1,6
33	St. 8 # 6	0,96	11	11	0,79	0,37	0,024	1,1	0,88	11	210	0,75	1,1	1,9	<0,01	0,4	0,033	0,89
34	St. 8 # 7	0,89	8,1	12	<0,15	0,22	0,004	1,9	0,69	6,2	210	2,2	<1,6	1,7	<LOD	0,12	<0,0047	0,45
35	St. 8 # 8	0,96	9,8	11	0,61	0,54	0,017	0,84	1,2	10	230	1,2	0,83	1,4	<0,01	0,24	0,019	0,59
36	NCS 7C 73014 tea	1,979	3,17	18,39	0,147	0,352	0,503	0,219	3,611	19,7	51,01	0,08	<LOD	0,07	0,033	1,41	0,023	0,009
37	IAEA 350 tuna	1,455	9,391	14,22	<0,02	1,093	6E-04	0,022	0,333	2,953	16,58	5,305	<LOD	0,011	<0,003	0,033	<0,0008	<0,002

Kommentarer:

Enkelte av prøvene hadde konsentrasjoner av Cd og Ba som var en del høyere enn standardene

Det er litt høy bakgrunn av Mo i blanken, derfor vet vi ikke om vi treffer på referansematerialet, så litt usikre tall her.

LOD		2E-05	0,002	6E-04	0,006	0,01	1E-05	5E-04	0,03	0,7	0,6	8E-04	0,002	2E-04	2E-04	0,008	2E-04	8E-05
LOQ		7E-05	0,007	0,002	0,019	0,038	4E-05	0,002	0,088	2,5	2,1	0,003	0,006	8E-04	8E-04	0,028	7E-04	3E-04
Dilution samples, mL	25	Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
		g/kg	g/kg	g/kg	mg/kg	mg/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
78	blank	<0,00007	<0,007	<0,002	<LOD	<LOD	<0,00004	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,0007	<LOD
79	blank	<0,00007	<LOD	<0,002	<LOD	<LOD	<0,00004	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,0007	<LOD
80	blank	<0,00007	<LOD	<0,002	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
118	blank	<0,00007	<LOD	<0,002	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
119	blank	<0,00007	<LOD	<0,002	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
120	blank	<0,00007	<LOD	<0,002	<LOD	<LOD	<0,00004	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,0007	<LOD
41 (1)	3.1.1	1,1	6,7	9,7	12	3,2	0,026	2,2	3,4	12	95	1,8	0,95	2,1	0,053	4,9	0,28	5,4

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
42 (2)	3.1.2	1,3	8	9,7	16	3,9	0,039	2,7	4,5	14	110	0,87	1,4	2,7	0,073	7,1	0,3	6,3
43 (3)	3.1.4	0,92	8,1	10	2,8	2,1	0,06	4	4,2	24	170	2,6	1,3	2,6	0,29	22	0,34	0,65
44 (4)	3.1.7	1,5	7,9	10	17	5	0,038	2,8	5,2	15	130	1,1	1,4	4	0,057	5,8	0,45	7,8
45 (5)	3.1.10	1,7	7,6	11	20	6,7	0,042	3	6,6	18	130	1,3	1,8	4,7	0,063	5,9	0,65	12
46 (6)	3.1.15	1,6	8,3	10	15	5,4	0,049	3	6,4	20	120	1,1	1,6	5,5	0,056	5,6	0,93	9,3
47 (7)	3.1.22	1,2	8,4	10	7,4	3,1	0,029	4,1	6,2	22	120	0,43	1,4	18	0,037	6,1	0,22	7,3
48 (8)	3.1.29	2	9	10	27	8,1	0,047	3,1	11	23	140	0,97	2,5	13	0,087	6,9	0,76	14
49 (9)	3.1.36	2,3	8,8	10	28	10	0,056	3,9	9,7	24	140	0,96	3,1	12	0,074	7,5	1,1	21
50 (10)	3.2.1	1,4	7,6	10	14	4,3	0,032	2,2	3,9	13	110	0,63	1,2	2,1	0,065	4,6	0,35	7,2
51 (11)	2.1.1	0,8	7,2	9	2	1,2	0,017	2,6	1,5	13	93	2,1	0,58	2,1	0,007	4,4	0,065	3,6
52 (12)	2.1.2	0,7	6,7	8,1	0,26	0,12	0,009	2,4	0,56	8,1	88	0,82	0,3	2	0,005	3,3	0,01	0,56
53 (13)	2.1.4	2,4	7	9,4	17	10	0,059	3,1	3,9	19	96	0,69	2,2	1,6	0,009	6,7	1,1	18
54 (14)	2.1.7	1,7	7,4	10	8,5	5,5	0,041	2,8	2,6	17	110	3,6	1,4	2,5	0,012	6,9	0,62	8,2
55 (15)	2.1.10	1,5	9,1	10	7	4,5	0,041	4,1	2,4	21	110	0,53	1,2	2,7	0,042	4,7	0,41	6,4
56 (16)	2.1.15	1,8	8,1	9,9	11	6,8	0,042	4,4	3	20	110	1,6	1,9	3,1	0,014	6,8	0,65	12
57 (17)	2.1.22	1,5	8,8	9,4	7,4	4,2	0,045	5,1	3,2	20	130	0,77	1,4	4,9	0,012	7,2	0,39	7,8
58 (18)	2.1.29	1,1	10	9,3	3	1,8	0,029	5,4	1,8	17	130	0,3	1,1	4,4	0,01	7,2	0,17	3,9
59 (19)	2.1.36	0,87	9,6	10	0,7	0,38	0,023	4,4	1,8	24	130	0,38	1,6	6,4	0,01	6,2	0,048	7,4
60 (20)	2.2.1	1,4	7,1	9,6	6,7	4,3	0,027	2,4	2,1	13	90	2,3	0,91	1,6	0,011	4,8	0,37	7,9
61 (21)	1.1.1	0,78	8,6	6,8	1,1	0,46	0,04	3,1	0,81	12	120	0,64	0,66	1,8	0,075	4,6	0,078	0,17
62 (22)	1.1.2	0,72	7,4	8,8	1,1	0,39	0,037	1,9	0,68	6,8	110	4,9	0,52	1,5	0,016	6,3	0,32	0,23
63 (23)	1.1.4	0,58	7,5	7,8	0,28	0,084	0,026	3,1	0,5	9,1	110	15	0,46	2,1	0,013	7,3	0,021	0,13
64 (24)	1.1.7	0,97	9,8	9,5	3,4	1,9	0,081	4,2	1,8	13	150	0,98	1,8	3,1	0,16	19	0,37	0,71
65 (25)	1.1.10	0,8	8,5	10	1,1	0,54	0,062	4,9	0,84	13	120	0,71	1,1	2,6	0,12	15	0,12	0,35
66	1.1.15	0,85	9	10	1,9	0,84	0,061	4,3	1,4	8,6	130	0,56	1,4	3,4	0,037	26	0,47	0,49
67	1.1.22	0,78	9,4	9,2	0,91	0,45	0,048	4,1	1,1	10	140	0,8	0,83	4,3	0,034	27	0,079	0,4
68	1.1.29	0,98	7,9	9,3	5,4	3,2	0,083	4,8	2,4	11	130	2,2	2,9	3,5	0,09	43	0,68	1,3
69	1.1.36	0,71	13	7,4	1,1	0,29	0,03	4,5	0,98	<12	120	0,29	0,75	4,3	0,021	13	0,067	0,42
70	1.2.1	0,78	7,3	8,7	1,5	0,77	0,034	2,6	0,98	7,5	100	0,94	1	1,7	0,22	6,8	0,15	0,3
71	4.2.1	0,76	7,3	8,6	1,2	0,22	0,015	2,3	2,6	12	110	1,9	0,66	3,1	0,02	5,3	0,022	2,2
72	1.2.4	0,7	8,4	3,4	0,98	63	0,047	4,9	1,2	12	130	0,35	0,89	3,1	0,028	10	0,11	0,35
73	2.2.4	2,6	7,1	10	17	12	0,065	3	4,4	17	110	0,78	2,1	1,9	0,008	8,1	1,3	24

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
74	3.2.4	2	7,6	9,8	27	8,8	0,049	3,3	6,4	16	120	2,9	1,9	3,1	0,062	6,6	0,82	12
75	4.2.4	1,2	8,5	9,9	30	3,2	0,031	3,4	10	16	140	1,7	1,8	7,7	0,16	5,7	0,39	7,2
81	1.3.1	0,78	8,2	8,8	1,1	0,54	0,047	3	0,81	12	110	0,46	0,82	1,5	0,077	5,6	0,12	0,27
82	1.4.1	0,71	8,6	8,1	0,22	0,087	0,024	2,6	0,54	7	110	0,44	0,4	1,7	0,014	6,4	0,023	0,11
83	1.5.1	0,84	7,2	8,9	2,8	1,7	0,071	3,1	1,5	11	110	0,5	1,6	2,3	0,051	10	0,31	0,65
84	2.3.1	1,3	7	9,3	6,7	4,1	0,032	2,6	2	13	100	2,2	0,93	1,7	0,011	5,6	0,38	8
85	2.4.1	1,3	7,8	10	5,5	3,5	0,031	3,4	2	17	110	0,59	0,94	1,9	0,064	5,1	0,3	6,1
86	2.5.1	1,9	7	9,2	11	7	0,049	2,9	2,9	17	110	1,3	1,7	1,7	0,014	5,5	0,68	12
87	3.3.1	0,59	6,3	9,1	0,11	0,053	0,007	3,1	1,2	11	87	4,7	0,3	2	0,007	3,9	0,004	1,6
88	3.4.1	1,4	6,9	7,8	14	4,8	0,036	2,6	3,6	13	91	1,6	1,2	1,8	0,045	4,9	0,46	8,6
89	3.5.1	0,73	8	6,8	0,52	0,38	0,031	2,1	1,5	19	150	0,95	0,49	1,8	0,04	6,7	0,029	0,94
90	4.3.1	1,2	8,1	9,3	22	3	0,021	2,1	7,8	16	120	0,85	1,6	3,5	0,15	5,9	0,28	6,2
91	4.4.1	0,82	8,8	9,2	2,4	0,47	0,013	2,3	3	11	110	1,1	0,52	2,5	0,028	6,6	0,041	2,8
92	4.5.1	0,87	7,2	9,1	19	1,6	0,018	2	5	9,6	89	1	0,97	2,6	0,11	4	0,14	3,7
93	1.2.2	0,68	8	8,8	0,59	0,25	0,036	3,1	0,56	8,3	100	0,41	0,62	1,8	0,085	6,3	0,059	0,2
94	1.3.2	0,95	8,4	8,9	3,4	1,9	0,067	3,8	1,7	12	130	0,73	1,7	2,3	0,075	12	0,43	0,67
95	1.4.2	0,69	7,7	9,2	1,1	0,6	0,025	2,7	0,95	8,8	97	0,46	0,83	1,9	0,03	10	0,13	0,35
96	1.5.2	0,89	8,9	8,9	2,9	1,4	0,052	4,3	1,4	13	110	2,1	1,7	2,1	0,11	13	0,42	0,64
97	2.2.2	1,7	6,2	8,5	10	6,9	0,042	2,7	2,8	13	95	14	1,3	1,2	0,012	5,2	14	11
98	2.3.2	0,95	7,1	8,9	3,3	2	0,026	2,8	1,2	12	110	10	0,66	1,7	0,01	4,3	0,16	3,2
99	2.4.2	1,6	7,4	9,5	8,3	5,5	0,036	3,2	2,4	14	110	0,47	1,2	2,4	0,013	6,6	0,49	7,5
100	2.5.2	0,78	7,2	8,9	0,83	0,45	0,016	2,3	1	12	100	0,4	0,46	2,1	0,006	4,8	0,034	3,1
101	3.2.2	1,5	7,7	9,6	22	5,3	0,033	3,3	4,8	15	100	0,85	1,6	1,9	0,035	5,4	0,49	9
102	3.3.2	2	7,2	10	22	8,1	0,043	3,3	6,1	17	100	1,2	2	2,5	0,051	6,1	0,85	16
103	3.4.2	0,75	8,9	7,6	0,65	0,23	0,02	4,6	0,96	9,5	110	0,45	0,46	2,1	0,041	4	0,025	0,9
104	3.5.2	1,3	8,1	10	14	4,3	0,031	4,1	3,6	11	120	0,63	1,4	2,8	0,14	7	0,33	6,9
105	4.2.2	1,4	8,6	10	46	4,5	0,028	2,5	9,9	17	110	1,3	1,9	3,5	0,14	5,9	0,41	9,8
106	4.3.2	1,2	8,4	10	22	2,6	0,03	2,5	8	13	130	2	1,5	5,6	0,13	6,4	0,26	7,6
107	4.4.2	2	8	10	91	9,7	0,046	3,2	20	19	120	3,2	3,9	4,3	0,23	8,3	1,1	20
108	4.5.2	1,2	7,9	9,7	33	3,1	0,026	2,6	9,2	14	120	2,7	1,7	4,4	0,17	6,3	1,2	7,8
109	1.3.4	0,84	7,7	9	2,8	1,4	0,083	3,4	1,2	10	110	0,61	1,5	1,8	0,073	16	0,5	0,8
110	1.4.4	0,7	7,3	8,7	0,97	0,4	0,047	2,2	1,1	7,4	110	0,39	0,78	1,2	0,023	9	0,1	0,31

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
111	1.5.4	0,59	6,9	8,2	0,16	0,32	0,018	2,2	0,47	5,3	91	0,43	0,33	1,4	0,013	7,4	0,007	0,11
112	2.3.4	1,7	6,4	9	9,7	6,2	0,042	2,5	2,6	14	92	0,7	1,3	1,8	0,011	5,8	0,71	12
113	2.4.4	0,98	8,1	10	3,8	1,6	0,015	2,9	3,9	9,9	96	0,39	0,57	2,9	0,025	7	0,07	3
114	2.5.4	1,6	7,8	10	8,4	6,7	0,039	3,6	3,4	17	100	0,56	1,4	2,7	0,015	6,8	0,56	9,9
115	3.3.4	2	7,2	9,9	25	8,5	0,048	2,7	6,3	14	110	0,92	2,1	2,6	0,06	7,4	0,9	16
121	3.4.4	0,99	7,1	8,7	8,5	2,2	0,022	3	3,1	14	100	0,71	0,81	2,9	0,038	4,1	0,19	4,1
122	3.5.4	0,89	7,4	9,1	7,8	1,6	0,023	2,1	3,2	14	96	0,96	0,65	2,4	0,035	4,2	0,2	3,5
123	4.3.4	0,89	7,4	9,9	17	1,8	0,018	2,9	7,2	11	110	0,66	1	7,3	0,11	4,4	0,12	6,7
116	NCS 7C 73014 tea	1,732	3,038	16,44	0,14	0,238	0,504	0,218	3,392	18,98	50,4	0,086	0,028	0,066	0,024	1,388	0,028	0,01
76	NCS 7C 73014 tea	1,764	3,117	16,29	0,152	0,241	0,517	0,223	3,39	19,33	51,66	0,082	0,03	0,067	0,023	1,397	0,028	0,009
77	IAEA 350 tuna	1,32	8,816	13,31	0,014	0,551	5E-04	0,021	0,243	2,411	17,02	5,046	0,014	0,01	0,001	0,03	6E-04	9E-04
117	IAEA 350 tuna	1,284	8,748	13,36	<0,01	0,529	5E-04	0,022	0,258	2,829	16,03	5,009	0,015	0,009	<0,0005	0,025	<0,0005	4E-04
LOD		0,002	0,003	0,006	0,02	0,04	4E-04	0,002	0,1	0,4	0,4	0,001	0,03	6E-04	0,001	0,03	0,09	0,004
LOQ		0,006	0,01	0,021	0,064	0,15	0,001	0,006	0,35	1,4	1,3	0,005	0,1	0,002	0,004	0,1	0,31	0,015
		Mg	**S**	**K**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sb**	**Pb**	**Th**	**U**
		g/kg	g/kg	g/kg	mg/kg	mg/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Sample Name	Comment																	
158	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
159	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,1	<LOD	<LOD	<LOD	<LOD	<LOD
160	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
198	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
199	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<1,4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
200	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
238	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
239	blank	<LOD	<LOD	<LOD	<0,06	<0,15	<0,0013	<0,006	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,1	<LOD	<0,015
240	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
277	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
278	blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
124	4.4.4	1,1	7	9,2	38	5	0,031	2,5	9,4	13	110	1,8	1,8	4,4	0,15	4,3	0,28	7,7
125	4.5.4	0,76	6,6	8,5	13	1	0,015	1,8	4,9	11	99	1,4	0,89	5,5	0,09	4,9	<0,3	4,4
126	1.2.7	0,79	8	9,4	1,7	0,69	0,046	3	44	7,1	120	0,75	1,4	2,7	0,045	16	<0,4	0,5

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
127	1.3.7	0,75	7,6	8,8	2,3	1,4	0,062	3,6	1,4	8,4	120	0,51	1,5	2,5	0,09	16	0,27	0,63
128	1.4.7	0,75	6,6	8,3	2,6	1,5	0,053	2,5	1,6	7,3	97	0,6	1,4	1,6	0,13	14	0,3	0,58
129	1.5.7	0,69	6,1	8,2	1,8	1,2	0,045	2	1,2	8	100	1,8	1,1	1,3	0,037	13	<0,3	0,46
130	2.2.7	2,5	6,5	9,9	18	13	0,055	4,3	4,5	16	94	0,51	2,6	3	0,009	8,3	1,4	19
131	2.3.7	1,3	9	9,9	5,9	4,3	0,027	3,4	2	12	110	0,8	0,87	2,4	0,025	5	0,38	6,1
132	2.4.7	1,4	7,5	10	6,9	4,8	0,034	3,3	2,5	14	100	0,39	1,2	2,8	0,014	6,3	0,45	11
133	2.5.7	2	6,5	9,9	13	9,6	0,044	2,7	3,2	13	91	1,8	1,8	1,7	0,015	5,4	0,98	14
134	3.2.7	2,4	7,6	9,8	27	12	0,058	3,8	8	17	120	0,84	2,5	4,3	0,062	8	1,3	17
135	3.3.7	1,5	7,7	9	15	5,8	0,048	3,3	5,3	24	120	0,73	1,6	4	0,11	7,5	0,63	9,6
136	3.4.7	0,99	7,8	9,3	5,3	2,2	0,03	2,8	3,3	16	110	0,4	0,87	5	0,028	4,3	<0,3	6,8
137	3.5.7	1	6,6	9	6,4	3	0,028	2,6	3,8	12	97	0,4	1	5,5	0,035	4,9	<0,4	5,5
138	4.2.7	1,3	8,2	9,7	35	4,3	0,028	3	12	17	130	1,3	2	11	0,18	6	0,41	9,3
139	4.3.7	0,92	8,5	9,4	11	1,7	0,032	3	7,1	16	120	1,4	1,2	8,8	0,12	4,9	<0,2	8,4
140	4.4.7	1,5	7,3	10	46	6	0,033	2,2	13	14	120	1,3	2,6	5,4	0,19	5,4	0,63	9,7
141	4.5.7	1,1	8,5	8,7	48	4,2	0,028	5,1	12	15	120	1,4	2,2	4,3	0,24	4,5	0,41	9,4
142	1.2.10	0,69	7,3	9	1,2	0,56	0,065	3,2	1	7,6	100	0,93	1,3	2,5	0,029	16	<LOD	0,34
143	1.3.10	0,84	7,4	9	2,7	2,7	0,064	2,6	2	8	120	1,3	1,9	2,1	0,063	21	0,34	0,72
144	1.4.10	0,89	8,2	10	3,3	2,1	0,061	4,6	1,9	7,1	130	0,65	2	2,9	0,077	27	<0,7	0,99
145	1.5.10	0,72	7,3	10	0,93	0,53	0,04	3,4	1,1	9,5	110	0,38	0,86	2,5	0,034	17	<LOD	0,34
146	2.2.10	1,7	7,8	11	10	6,9	0,038	3,5	2,8	15	110	4,2	1,6	2,5	0,012	6,4	0,66	12
147	2.3.10	1	7,2	10	3,3	2,1	0,026	3	1,5	12	100	0,37	0,9	2,6	0,01	3,8	<0,4	5
148	2.4.10	1,4	7,9	9,7	6,3	4,4	0,044	4,7	2,2	15	120	2,3	1,2	2,7	0,011	5,8	0,41	7,3
149	2.5.10	1,7	7,5	9,7	8,7	6,1	0,043	4,6	2,7	19	110	0,54	1,6	2,8	0,057	6,7	0,58	10
150	3.2.10	1,3	8,1	9,8	10	3,5	0,036	3,5	4,7	14	110	0,93	1	7,4	0,043	6	<0,3	4,8
151	3.3.10	1,7	7,1	9,1	18	6,9	0,044	3,4	7,2	16	120	1,8	2	6,6	0,06	7,7	0,73	12
152	3.4.10	1,7	7,7	8,9	22	6,8	0,042	4,1	5,7	16	120	0,96	1,9	2,5	0,13	6,6	0,64	9,6
153	3.5.10	1,7	7	9,4	20	7	0,041	3,1	7,2	15	110	0,66	1,9	6,8	0,062	7,3	0,75	11
154	4.2.10	1,4	9,3	9,3	31	4,8	0,041	3,6	13	17	130	1,1	1,9	5,5	0,18	6,1	<0,5	8,2
155	4.3.10	0,83	7,3	8,5	21	1,5	0,021	2,1	6,9	13	110	0,97	1,2	6,3	0,1	4,1	<0,3	3,3
161	4.4.10	1,1	9,4	10	20	2,1	0,024	4,7	8,2	19	120	0,78	1,3	6,7	0,15	4,8	<0,3	5,7
162	4.5.10	1,2	7,6	9,8	30	4	0,024	2,4	13	13	130	1,2	3,2	13	0,26	4,7	<0,6	10
163	1.2.15	0,74	8,1	9,5	0,44	0,11	0,056	3,2	0,75	9,2	120	0,64	0,88	3,7	0,062	18	<LOD	0,29

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
164	1.3.15	0,84	7,6	9,1	3,3	1,4	0,058	3,6	1,3	9,5	110	0,5	1,7	3,5	0,052	24	0,43	0,7
165	1.4.15	0,83	8,7	9,6	1,7	0,57	0,043	4	1,5	7,6	150	1,2	1,6	3	0,029	33	<LOD	0,47
166	1.5.15	0,69	8,3	9,2	0,5	0,19	0,034	2,6	0,6	6,6	120	0,67	0,64	2,4	0,035	21	<LOD	0,25
167	2.2.15	1,3	9,2	9,2	5,6	4,1	0,023	3	2,1	15	120	0,62	1,2	2,3	0,11	5,2	<0,4	6,9
168	2.3.15	1,1	8	8,9	4,4	3,1	0,023	2,9	1,6	22	150	1,8	0,95	2,2	0,014	4,7	<0,3	5,2
169	2.4.15	1,4	8,5	9,7	6,6	4,5	0,034	3,8	2,5	17	130	0,55	1,3	2,9	0,06	6,3	0,35	8,4
170	2.5.15	1,7	9,3	9,7	9,9	6,9	0,046	4,8	3,5	22	120	0,59	2,1	3,4	0,075	8,9	0,65	14
171	3.2.15	1,8	8,9	11	18	7,4	0,044	3	7,2	17	120	0,54	1,9	6,1	0,063	5,2	0,78	11
172	3.3.15	1,1	8,7	9,6	5,7	2,2	0,034	3,9	3,8	16	130	0,7	1,3	6,2	0,097	4,9	<0,2	5,4
173	3.4.15	1,6	7,9	10	24	6,8	0,061	3,9	7,6	18	130	0,88	1,9	9	0,071	5,9	0,74	11
174	3.5.15	1,5	9,2	10	15	4,5	0,04	3,1	5,7	13	120	0,7	1,5	6	0,064	5,9	<0,5	6,3
175	4.2.15	1,2	8,2	8,5	61	4,2	0,04	3,9	13	18	120	2,3	2,6	13	0,19	4,9	0,42	8,5
176	4.3.15	1	8,4	8,2	3	16	0,081	3,4	12	100	400	1,8	1,9	2,3	0,32	13	<0,5	0,27
177	4.4.15	1,4	8,3	9,9	65	6,3	0,035	3,2	17	19	130	1,9	2,9	24	0,22	7,3	0,6	9,2
178	4.5.15	1,6	9,8	10	72	7	0,038	3,6	19	20	140	1,9	2,8	5,7	0,26	5	0,76	13
179	1.2.22	0,9	7,2	9,3	3,6	2	0,055	2,3	1,7	7,6	120	1,3	1,8	1,7	0,067	31	0,36	0,85
180	1.3.22	0,69	8	9	0,72	0,36	0,033	3,2	0,84	8,3	110	1,5	0,71	2,7	0,025	19	<LOD	0,35
181	1.4.22	0,79	7,6	9	1,9	0,99	0,044	4,4	1,4	7,2	120	0,74	1,4	3,4	0,038	32	<0,3	0,48
182	1.5.22	0,75	9,4	8,6	1,4	0,74	0,046	4,2	1,1	8,4	140	0,57	0,9	4	0,032	35	<LOD	0,58
183	2.2.22	0,96	8,8	10	2,1	1,3	0,029	3,4	2	17	110	0,3	0,94	3,8	0,01	3,9	<0,5	5,7
184	2.3.22	1,5	8,4	9,9	7,5	5	0,038	4	3	21	110	0,61	1,5	4,3	0,015	6,6	0,46	13
185	2.4.22	1,2	8,7	9,4	5,3	3,1	0,04	3,7	2,8	20	120	0,92	1,2	4	0,015	4,1	<0,3	8,4
186	2.5.22	1,1	9,1	7,7	3,6	2,5	0,031	4	1,9	20	130	0,61	0,77	4,6	0,013	4,6	<0,3	5,8
187	3.2.22	1,2	9,5	9,9	12	2,9	0,032	3,7	8,9	20	130	1,1	1,6	15	0,091	6	<0,4	5,7
188	3.3.22	1,6	8,6	9,8	24	6,7	0,041	4,7	9,5	19	120	0,78	1,9	11	0,067	5,8	0,56	12
189	3.4.22	1,3	7,3	8,9	14	4,9	0,032	3,2	5,8	16	120	10	1,6	6,5	0,012	4,9	0,45	9,1
190	3.5.22	0,86	8,3	10	1,9	0,81	0,024	3,9	4,9	17	120	0,42	1,1	11	0,02	3,7	<LOD	5,1
191	4.2.22	1,2	11	11	33	2,6	0,028	2,4	14	24	140	1,2	2	23	0,14	4,6	<0,2	9
192	4.3.22	0,97	9,7	10	5,1	0,91	0,024	2,4	12	21	150	0,83	1,1	24	0,14	5,5	<LOD	9,5
193	4.4.22	1,2	9,1	10	37	3,5	0,022	3	17	22	160	1,4	2,3	26	0,19	6,3	<0,4	13
194	1.2.29	0,91	7,5	9,6	4,1	2,6	0,07	3,6	1,9	8,1	120	2,1	2,2	2,9	0,074	43	0,54	0,94
195	1.3.29	0,83	9,2	10	2,1	1	0,042	4,6	1,3	6,6	120	0,75	1,4	4,4	0,049	56	<LOD	0,68

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
201	1.4.29	0,93	8,7	10	3,8	2,3	0,054	5,1	2,1	7,4	120	2,3	1,8	3,7	0,067	53	<0,4	0,86
202	1.5.29	0,78	9,2	9,5	1,5	0,74	0,082	5,2	1,4	8,7	130	0,64	1,2	4,6	0,11	47	<0,3	0,41
203	2.2.29	1,8	8,7	11	9,6	6,5	0,047	3,6	3,1	21	130	0,82	1,8	3,7	0,013	6,4	0,61	10
204	2.3.29	1,5	8,6	9,7	7	4,6	0,032	3,5	2,9	20	120	0,8	1,5	3	0,033	6,7	0,39	11
205	2.4.29	1,6	8,7	10	8,7	5,4	0,039	4,4	2,7	17	130	0,43	1,5	5,5	0,019	5,9	0,48	10
206	2.5.29	1,3	8,8	9,4	5,8	4	0,04	5,9	2,7	21	100	0,57	1,6	4,3	0,16	5,7	0,45	8,9
207	3.2.29	1,5	9,2	10	27	5,1	0,044	3,4	9,3	20	120	1,3	2,1	16	0,072	6,4	0,47	8,1
208	3.3.29	1,1	9,9	9,3	14	2,9	0,031	3,7	7,9	21	130	0,88	1,4	18	0,051	5,4	<0,4	13
209	3.4.29	1,8	8	11	19	6,8	0,052	4	8,7	20	130	0,86	2,2	12	0,065	6,3	0,69	14
210	3.5.29	1,2	9,6	11	7,6	2,6	0,026	4,3	5,9	19	130	0,54	1,3	22	0,043	5,5	<0,5	6,6
211	4.2.29	1,2	9,2	10	38	3,8	0,027	3,7	14	21	140	1,7	2,5	23	0,22	6,3	0,38	8,9
212	4.3.29	1,2	9,8	11	28	3,3	0,027	2,9	15	21	140	0,98	1,8	52	0,16	4,6	<0,5	12
213	1.2.36	0,96	8,5	9,4	3,5	2	0,071	3,7	2,1	10	140	0,87	2,3	6	0,081	59	<0,4	0,99
214	1.4.36	0,98	8,3	10	4,5	2,1	0,055	3,7	2,1	8,5	120	0,95	2,7	4,1	0,079	94	<0,6	1,2
215	1.5.36	0,81	8,1	9,5	2,2	1,2	0,049	4,8	1,5	12	110	1,1	1,8	2,8	0,12	47	0,25	0,76
216	2.2.36	2,7	7,8	9,7	17	12	0,062	4,2	4,8	23	90	0,89	2,6	4	0,046	10	1,1	18
217	2.3.36	0,99	10	10	0,93	0,55	0,023	4,4	1,3	22	110	0,37	0,82	5,1	0,022	3,6	<LOD	3,1
218	2.4.36	1	9,8	9,8	4,3	1,6	0,019	3	2	17	130	1,1	0,97	3,7	0,017	6,6	<LOD	4,2
219	2.5.36	1,6	7,1	9,4	8,6	6	0,045	4,7	2,8	17	100	2	1,3	3,3	0,01	4,4	0,6	7,7
220	3.2.36	2,4	8,8	10	28	11	0,052	5,1	12	29	130	1,2	3,4	14	0,11	8,4	1,1	22
221	3.3.36	1,3	9	9,7	15	4	0,034	4,5	7,5	22	110	0,7	1,8	18	0,11	4,3	0,35	8,1
222	3.4.36	1,7	9	11	22	6,5	0,042	4,4	8,9	20	120	0,84	2,1	18	0,061	6,7	0,65	13
223	3.5.36	2	7,1	5,5	28	8,8	0,056	3	8,2	23	150	0,97	2,4	10	0,093	12	0,88	16
224	4.2.36	1,8	9,7	10	49	7,1	0,038	3,6	19	24	140	1,6	3,2	52	0,22	7,4	0,77	17
225	4.3.36	1,6	9,8	10	47	5,4	0,036	4,6	19	27	140	1,7	3	25	0,23	6,9	0,5	13
226	1.1.70	0,75	8,8	7,3	1,2	<0,49	0,022	6,2	1,4	8	120	3,1	1,5	6,7	0,063	140	<LOD	0,91
227	1.2.70	0,93	9,6	9,5	3,3	1,7	0,045	4,9	2	8,5	140	0,99	2,5	12	0,072	150	1,2	1,1
228	1.3.70	0,97	9,5	9,3	3,7	2,2	0,066	4,1	1,9	7,9	150	3	2,5	6,1	0,08	170	<0,5	1,1
229	1.4.70	0,79	10	9,8	1,5	0,81	0,051	3,6	1,7	7,9	110	1,4	2,4	8,7	0,06	140	<0,4	0,77
230	1.5.70	0,91	9,8	9,4	2,5	1,1	0,047	4,3	1,8	8	140	0,8	2,2	12	0,066	120	<0,6	0,95
231	2.1.70	1,3	9,4	9,3	5	3,2	0,033	6,1	2,6	20	130	0,48	1,6	9	0,016	7,8	<0,5	13
232	2.2.70	2,2	8,9	9,5	14	8,9	0,051	5,8	4,4	28	110	0,69	2,6	6,5	0,01	6,8	0,87	26

		Mg	S	K	V	Cr	Mn	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Pb	Th	U
233	2.3.70	1,7	11	9,4	8,4	5,6	0,039	6,3	3,4	20	130	0,44	2	9,4	0,018	8,6	0,48	16
234	2.4.70	1,4	10	9,9	6,7	4,3	0,034	5,2	2,5	18	130	0,47	1,6	10	0,017	6,7	<0,4	10
235	3.1.70	1,5	9,6	9,6	15	4,8	0,032	4,7	9,9	19	140	0,96	2,5	27	0,12	6,4	0,4	15
241	3.2.70	1,4	10	9,9	16	4,2	0,039	5,3	7,7	19	150	0,8	2,1	50	0,065	5,6	<0,4	12
242	3.3.70	1,4	9,3	9,3	14	4,4	0,034	5,1	7,1	14	130	0,77	2,2	37	0,14	5,6	0,33	13
243	3.4.70	1,6	10	10	15	5,6	0,039	5,2	8,6	25	130	0,71	2,2	52	0,068	5,2	<0,5	14
244	3.5.70	1,3	9,7	8,7	9,3	3,8	0,043	6,3	6,4	18	140	0,61	2	29	0,14	4,7	0,32	12
245	4.1.70	2	11	10	75	8,2	0,043	5,9	27	25	160	2,3	4,3	54	0,32	8	0,76	16
246	4.2.70	1,4	11	11	31	3,6	0,051	4,3	20	24	160	1,3	3,1	100	0,21	5,6	<0,3	14
247	3.1.71	1,4	9,8	9,6	12	4	0,031	5	8,1	26	140	0,55	2,3	39	0,063	5,8	<0,4	10
248	cocoons 3.1 (10)	0,68	7,7	2,6	3	1,5	0,027	0,43	2,4	22	250	0,069	0,52	5	0,12	0,62	<LOD	9,8
249	cocoons 3.3 (14)	0,63	6,5	1,3	3,5	1,2	0,025	0,67	4,7	19	210	0,094	0,47	4,9	0,14	0,52	<0,6	7,6
250	cocoons 3.4 (7)	0,88	7	2,1	7,1	3,6	0,038	0,55	3,8	19	180	0,14	0,89	4,3	0,17	1,4	<LOD	12
251	dried horse manure	2,7	2,1	15	6	3,9	0,45	0,96	4,2	25	110	0,24	2,1	0,17	0,021	1,2	0,97	0,28
156	NCS 7C 73014 tea	1,708	2,93	15,75	0,205	0,355	0,493	0,204	3,249	20,61	52	0,081	<0,07	0,067	0,035	1,569	<LOD	0,014
196	NCS 7C 73014 tea	1,689	3,017	15,98	0,141	0,281	0,492	0,202	3,279	18,45	52,79	0,076	<0,07	0,065	0,02	1,499	<LOD	0,01
236	NCS 7C 73014 tea	1,736	3,127	16,77	0,156	0,302	0,503	0,219	3,274	18,73	51,9	0,085	<0,06	0,067	0,028	1,539	<LOD	0,013
275	NCS 7C 73014 tea	1,7	3,065	16,21	0,168	0,282	0,503	0,214	3,226	18,67	51,36	0,083	<0,06	0,067	0,022	1,443	<LOD	0,012
157	IAEA 350 tuna	1,259	8,346	13,39	<LOD	0,57	<0,0009	0,022	0,383	2,921	16,08	4,83	<LOD	0,008	<LOD	<0,07	<LOD	<LOD
197	IAEA 350 tuna	1,285	8,282	13,88	<0,04	0,645	<0,0008	0,02	0,265	2,847	16,3	4,971	<LOD	0,012	<0,002	<0,06	<LOD	<LOD
237	IAEA 350 tuna	1,321	8,858	13,9	<0,04	0,824	<0,0008	0,021	0,3	2,883	16,05	5,095	<0,07	0,012	<0,002	<0,06	<LOD	<LOD
276	IAEA 350 tuna	1,232	8,547	13,2	<0,04	0,504	<0,0009	0,019	0,298	2,614	15,18	4,933	<LOD	0,013	0,003	<0,07	<LOD	<LOD

Kommentarer: Jeg vet ikke hvor godt Mo og Th treffer på standardene ettersom de ligger nær eller under Limit of detection og Limit of Quantification, så disse tallene er litt usikre.

Soil

Limit of detection, LOD in (w/V) and (w/w)			0,0005	0,001	0,004	0,03	0,04	0,0001	0,003	0,005	0,2	0,1	0,002	0,0008	0,0002	0,003	0,006	0,007
Limit of quantification, LOQ in (w/V) and (w/w)			0,0016	0,0032	0,012	0,094	0,13	0,0004	0,0085	0,017	0,52	0,33	0,0062	0,0027	0,0005	0,0091	0,019	0,024
Based on average weight, g		0,214656522																
Dilution samples, mL		10	**Mg**	**S**	**K**	**V**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Mo**	**Cd**	**Sb**	**Pb**	**U**
			g/kg	g/kg	g/kg	mg/kg	mg/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Sample Name	weight, g	Comment																
279	0,214656522	Blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,09	4,2	4,5	<LOD	<LOD	<0,003	<LOD	0,12	<0,12
280	0,214656522	Blank	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<0,09	4,6	4,3	<LOD	<LOD	<0,003	<LOD	0,13	<LOD
261	0,2271	soil 1.1	1,7	2	11	42	23	0,49	5,4	12	12	83	2,4	17	1	0,67	62	7
271	0,2295	soil 1.2	1,9	2,1	10	46	26	0,49	5,8	13	14	91	2,8	18	1,1	0,72	68	7
260	0,1998	soil 1.3	1,7	2	11	40	24	0,48	5,6	12	14	91	3	18	1,1	0,8	61	7
253	0,1883	soil 1.4	2,1	2,2	9,9	39	25	0,46	5,7	12	15	88	2,4	17	1,1	0,74	66	6,8
267	0,2312	soil 1.5	1,6	2,3	9,2	39	22	0,56	6,1	12	13	89	2,9	17	1,1	0,76	68	7,2
263	0,2219	soil 2.1	7,1	1,1	11	110	66	0,26	7	22	35	68	1,6	11	0,27	0,12	22	77
266	0,1981	soil 2.2	7,3	1	12	98	65	0,23	5,9	18	31	64	1,3	8,8	0,22	0,11	20	68
265	0,22	soil 2.3	7	1,1	13	100	65	0,24	6,1	19	32	64	1,4	9,3	0,25	0,12	20	71
262	0,2538	soil 2.4	7,3	1,1	12	110	65	0,28	6,5	19	33	66	2,2	10	0,28	0,12	22	78
254	0,2433	soil 2.5	8	1,3	12	100	67	0,26	6,6	20	34	67	1,7	11	0,31	0,14	23	82
259	0,1954	soil 3.1	6,7	3,1	13	350	67	0,25	7,6	55	41	120	8,6	17	1,7	1,8	24	75
258	0,207	soil 3.2	7,3	3,1	12	340	68	0,29	7,8	56	43	120	7,5	17	1,4	1,7	23	76
252	0,2255	soil 3.3	7,6	3,7	14	410	71	0,29	8,5	65	44	130	9,6	20	1,7	2,2	23	80
264	0,1846	soil 3.4	7	6,9	13	330	66	0,29	8,1	61	44	120	9,2	16	1,6	2	24	79
257	0,2296	soil 3.5	6,7	2,9	13	320	68	0,24	8	52	40	110	8,4	18	1,3	1,6	23	68
255	0,2075	soil 4.1	6,5	7,1	16	790	79	0,28	10	150	60	270	18	32	4,9	4,5	26	71
268	0,2791	soil 4.2	5,5	12	18	1000	77	0,31	12	180	70	300	28	40	6,3	6,8	30	84
270	0,2581	soil 4.3	5,5	11	18	1100	77	0,3	13	180	73	290	32	46	5,6	7	30	80
256	0,1802	soil 4.4	5,7	9,3	17	870	71	0,26	11	160	64	290	27	35	5,8	6	27	67
269	0,1916	soil 4.5	7,3	11	19	1200	78	0,57	12	180	95	330	29	39	6,5	6,4	29	78
272	0,1945	soil peat	0,86	1,6	2,1	3	1,7	0,092	0,58	1,3	14	46	0,95	1	0,29	0,28	15	<0,13
273	0,186	NCSDC73324	0,6893	0,2595	12,94	124,6	42,37	1,402	6,486	54,09	414	93,47	217,2	20,33	0,1423	63,37	309,2	5,954
274	0,185	2709a	11,25	0,5184	17,09	104	123,4	0,523	12,32	77,67	36,8	98,67	8,928	1,531	0,3354	1,495	16,85	3,011

6.2 Appendix 2 – Concentration ratios at day 70

Element	Tr. 1	1σ	Tr. 2	1σ	Tr. 3	1σ	Tr. 4	1σ	Orrefjell st. 7	1σ	Cocoons	1σ
Mg	0.48	0.11	0.22	0.07	0.20	0.03	0.28	0.11	0.19	0.11	0.10	0.02
S*	4.50	0.49	8.77	1.66	2.47	1.13	1.09	0.27	8.28	1.87	1.80	0.92
K	0.89	0.17	0.79	0.07	0.73	0.08	0.60	0.08	1.15	0.18	0.15	0.06
V	0.06	0.03	0.08	0.04	0.04	0.01	0.05	0.04	0.03	0.05	0.01	0.01
Cr	0.06	0.03	0.08	0.04	0.07	0.01	0.08	0.05	0.04	0.05	0.03	0.02
Mn	0.09	0.04	0.15	0.04	0.14	0.03	0.14	0.07	0.27	0.26	0.11	0.04
Co*	0.81	0.21	0.91	0.14	0.67	0.10	0.44	0.14	0.16	0.09	0.07	0.02
Ni*	0.14	0.02	0.16	0.06	0.14	0.04	0.14	0.04	0.05	0.06	0.06	0.03
Cu	0.59	0.07	0.65	0.17	0.45	0.11	0.34	0.07	0.35	0.18	0.47	0.06
Zn	1.49	0.24	1.90	0.20	1.15	0.14	0.54	0.07	5.97	2.48	1.75	0.40
As	0.69	0.48	0.32	0.14	0.09	0.02	0.07	0.04	0.15	0.11	0.01	0.01
Mo**	0.13	0.03	0.19	0.07	0.13	0.02	0.10	0.04	0.10	0.09	0.04	0.02
Cd*	8.43	2.96	32.8	9.9	25.3	10.5	13.2	7.0	30.1	19.2	3.07	0.61
Sb	0.09	0.02	0.13	0.04	0.06	0.03	0.04	0.02	0.11	0.10	0.08	0.02
Pb*	2.22	0.39	0.35	0.06	0.24	0.03	0.24	0.08	0.04	0.04	0.04	0.02
U	0.14	0.02	0.22	0.11	0.17	0.03	0.20	0.04	0.07	0.08	0.13	0.04

6.3 Appendix 3 – earthworm mortality

Number of replicates per treatment with surviving adult earthworms at each sampling point.

Day	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	5	5	5	5
2	5	5	5	5
4	5	5	5	5
7	5	5	5	5
10	5	5	5	5
15	5	5	5	5
22	5	5	5	4
29	5	5	5	3
36	5	5	5	3
70	5	4	5	2
Deceased worms	0	1	0	8

6.4 Appendix 4 – Orrefjell stations 5, 7 and 8 soil parameters

Soil parameters of soil sampled in situ at Orrefjell *According to Øien & Krogstad 1987

	pH	% DM	% LOI	Estimated %organic C*
Station 5	6.0	84.0	37.1	21.5
Station 7	4.5	97.2	23.0	13.3
Station 8	6.0	95.8	71.0	41.2

6.5 Appendix 5 – earthworm body weights and uncertainties

treatment	1	2	3	4	1stdev	1	2	3	4
1	1,19	1,07	1,03	0,77	1	0,62	0,32	0,53	0,17
2	1,17	0,94	1,00	0,95	2	0,29	0,34	0,56	0,44
4	0,99	0,98	0,85	1,00	4	0,51	0,51	0,39	0,26
7	1,05	0,85	0,88	1,14	7	0,27	0,13	0,42	0,43
10	0,92	0,98	0,88	0,89	10	0,35	0,25	0,18	0,38
15	0,89	0,99	0,88	0,93	15	0,28	0,14	0,33	0,78
22	0,71	0,78	1,08	0,78	22	0,28	0,14	0,54	0,40
29	0,88	0,96	0,78	1,12	29	0,33	0,70	0,18	0,38
36	0,71	0,84	1,09	0,78	36	0,46	0,25	0,51	0,13
70	0,60	0,89	0,97	1,07	70	0,24	0,50	0,28	0,07

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