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# Transfer of elements into boreal forest ants at a former uranium mining site<sup>☆</sup>

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## ABSTRACT

Ants can influence ecological processes, such as the transfer of elements or radionuclides, in several ways. For example, they redistribute materials while foraging and maintaining their nests and have an important role in terrestrial food webs. Quantitative data of the transfer of elements into ants is needed, e.g., for developing improved radioecological models. In this study, samples of red wood ants (genus *Formica*), nest material, litter and soil were collected from a former uranium mining site in Eastern Finland. Concentrations of 33 elements were analyzed by Inductively Coupled Plasma-Mass Spectroscopy/Optical Emission Spectroscopy. Estimated element concentrations in spruce needles were used as a proxy for studying the transfer of elements into ants via aphids because spruces host the most important aphid farms in boreal forests. Empirically determined organism/medium concentration ratios (CRs) are commonly used in radioecological models. Ant/soil CRs were calculated and the validity of the fundamental assumption behind the use of CRs (linear transfer) was evaluated. Elements that accumulated in ants in comparison to other compartments were cadmium, potassium, phosphorus, sulfur, and zinc. Ant uranium concentrations were low in comparison to soil, litter, or nest material but slightly elevated in comparison to spruce needles. Ant element concentrations were quite constant regardless of the soil concentrations. Non-linear transfer models could therefore describe the soil-to-ant transfer better than conventional CRs.

## 1. Introduction

The understanding of the transfer of elements and radionuclides in ecosystems is essential in order to assess risks from radioactive releases into the environment (Gilbin et al., 2021). Ants are organisms present almost everywhere in terrestrial ecosystems and can influence ecological processes, such as the transfer of elements or radionuclides, in several ways (Grześ, 2010a; Frouz et al., 2016).

Red wood ants (*Formica rufa* group) are common ant species in forests of the boreal area (Punttila & Kilpeläinen, 2009; Sorvari, 2021). They build large perennial nest mounds (volumes typically from 0.3 to 1.0 m<sup>3</sup>) using needles, twigs, branches, resin, and soil particles (Frouz et al., 2016). While constructing and maintaining the mounds, ants forage materials from a wide area (usually within 30 m distance from the

nest) and thus affect the distribution of elements in the environment (Niemelä and Laine, 1986; Frouz et al., 2016). For example, nest materials are rich in metals in metal-contaminated industrial area (Skaldina et al., 2018), and the accumulation of elements in the mounds can lead to the depletion of nutrients in the vicinity of the nests (Jílková et al., 2011). The nests contain belowground galleries with volumes similar to the aboveground parts (Frouz et al., 2016). When constructing these structures ants can transport soil with elevated metal concentrations from deeper soil layers to the soil surface (Frouz et al., 2016).

Ants play a major role in the transfer of elements and radionuclides in the food chain. Red wood ants consume honeydew produced by aphids and prey insects and other invertebrates (Frouz et al., 2016). On the other hand, they are prey of many organisms, such as bears, wild boars, and some bird species (Robinson et al., 2016). The competition

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between vertebrates and ants for invertebrate prey can also affect food web structures in forests (Jääntti et al., 2007; Robinson et al., 2016).

Ants can alter the transfer of elements also by affecting the functioning of the plants. As plants have a key role in cycling of elements, even small changes in plant growth and species distribution can be significant. The mutualistic relationships between ants and aphids can increase the number of aphids and decrease the number of other insects (Domisch et al., 2016). The effects of this relationship on plants depend on the insect species affected and the amount of sap removed by aphids (Kilpeläinen et al., 2009; Domisch et al., 2016). Ants can also distribute plant seeds to new places while moving around on soil surface (Robinson et al., 2016).

Red wood ants are known to accumulate metals and metalloids such as arsenic, cadmium, lead, nickel, and zinc (Starý and Kubizňáková, 1987; Rabitsch, 1995; Eeva et al., 2004; Grześ, 2010a; Skaldina et al., 2018). Honeydew can contain elevated concentrations of certain metals (e.g., cadmium), because aphids eliminate unnecessary substances via that route (Dar et al., 2017). It can therefore be an important pathway for the transfer of metals into ants (Starý and Kubizňáková, 1987). There are only few studies investigating the transfer of radionuclides to red wood ants (Dragović and Mandić, 2010; Dragović et al., 2010; Rosén et al., 2018) and to ants in general (Dragović and Mandić, 2010; Dragović et al., 2010; Mietelski et al., 2010; Medley et al., 2017).

Because ants are an important part of terrestrial ecosystems, more data on their role in the transfer of elements and their radionuclides is needed. Improved understanding of such transfer could help to identify and quantify the most important processes influencing radionuclide behavior, which is one of the key scientific challenges raised by the European Radioecology Alliance (Gilbin et al., 2021). This would be useful for the development and implementation of process-based radioecological models in the future (Hinton et al., 2013; Gilbin et al., 2021). New data related to ants is needed also from the point of view of existing radioecological models, which are important tools in radiation risk assessment. The models widely utilize concentration ratios (CRs) between organisms and the surrounding medium to describe the transfer of radionuclides in the environment (Hinton et al., 2013; IAEA, 2014; Raskob et al., 2018). Specific species are represented by broader groups in the models, for example ants belonging to the group of arthropods (Brown et al., 2016). Even for this generic group, the availability of data is quite limited for many radionuclides (Howard et al., 2013; IAEA, 2014). Data on the transfer of stable elements can be utilized in the development of radioecological models because the transfer of stable and radioactive isotopes of the same element is generally assumed to be similar (IAEA, 2010).

To gain insights into the transfer of elements into ants, the concentrations of 33 elements in ants, nests, litter, and soil were investigated in the vicinity of a former uranium mine in Eastern Finland. Ants representing *Formica rufa*-group (red wood ants) were collected as they are typical species in boreal forests and were abundant at the study site. The elements studied included necessary nutrients as well as elements having radioactive isotopes important from the point of view of radiation protection. The main objectives were to compare element concentrations in different compartments studied, to produce ant-to-soil CR values and to test whether there is a linear relationship between soil and ant concentrations. All these contribute to the underlying main aim of developing improved radioecological models by producing data needed for current models (ant-to-soil CR values) as well as increased understanding of the transfer of elements within boreal forest ecosystem.

## 2. Materials and methods

### 2.1. Study site

The study site was a former pilot-scale uranium mine located in Joensuu, Eastern Finland (N 6981372, E 653304, ETRS-TM35FIN) (Fig. 1). The area belongs to the central boreal forest zone. The

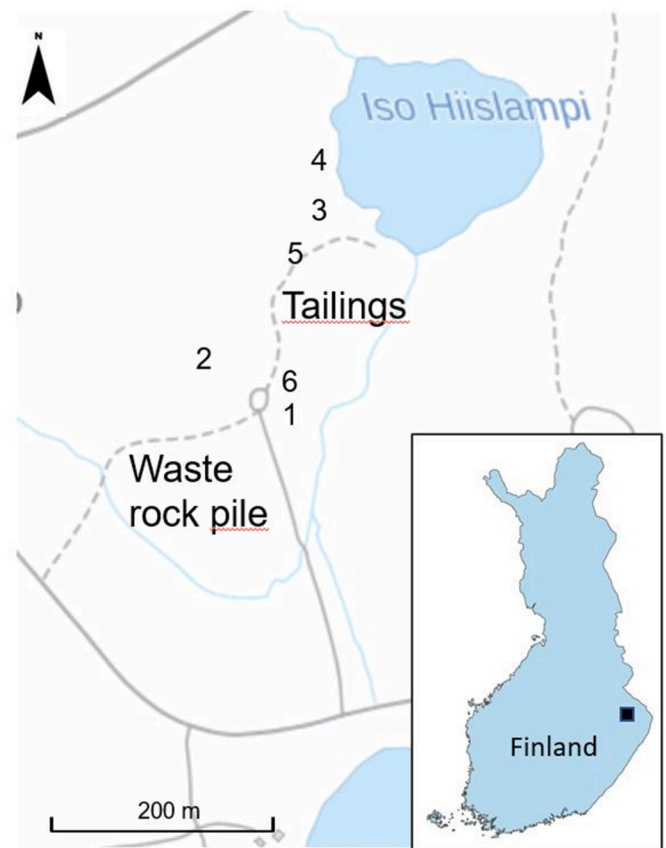


Fig. 1. Location of the former mining area in Eastern Finland and locations of the nest mounds within the area. Contains 1/2014 topographic data from National Land Survey of Finland (CC 4.0 BY).

average annual temperature is 2–3 °C, and there is a distinct difference in temperature between warmest (approximately 17 °C) and coldest (approximately –9 °C) months (Kersalo and Pirinen, 2009). The annual precipitation in the area is 550–650 mm and the length of the growing season approximately 160 days (Kersalo and Pirinen, 2009). The mine was in operation from 1959 to 1961 (Colpaert, 2006). The site was remediated in early 1990s by covering the waste-rock pile and the tailings with clay and till (Colpaert, 2006). The area is now considered suitable for outdoor use without restrictions, and it represents a typical boreal forest site (Colpaert, 2006). More information on the current state of the site is available from Tuovinen et al. (2016a, 2019).

### 2.2. Sampling

A survey was carried out at the site in early June 2020 to find the nest mounds existing in the vicinity of the former mining area. In total, samples were taken from six nest mounds. Nest mounds 1, 2 and 6 were located near the waste rock pile, mounds 3 and 4 near the shoreline of a small pond close to the tailings area, and mound 5 near the tailings area (Fig. 1). Ants were identified using the key to Collingwood (1979). Ant species sampled were *Formica polyctena* (nest 1), *Formica aquilonia* (nests 2–5) and *Formica rufa* (nest 6). All these species belong to the *Formica rufa*-group and their lifestyles are similar.

Samples of ants, nest material, decomposed nest material, litter and soil were collected on June 24th, 2020. The sampling time was selected based on the observations that metal levels in ants are elevated in summer when compared to spring (Rabitsch, 1995, 1997). Ants were sampled manually. A hand covered by a polyethylene glove was placed onto the surface of each mound and ants attached to the glove were transferred to plastic bags. Thus, only individuals moving outside the

nest (foraging workers) were collected. Our samples may represent workers with the greatest element concentrations in each study nest because foragers have been shown to have elevated concentrations of cadmium when compared to inside nest workers (Martin and Nuorteva, 1997). One composite sample of ants was collected from each nest. The fresh mass of ant samples varied from 8.4 g to 19 g. The ants were freeze-killed before weighing. Three sub-samples of nest surface material (fresh weights 14–31 g) at different heights of the mound (from the top, from the middle section and from the bottom just above the decomposed layer) were collected by a shovel from each mound. These sub-samples were combined into one sample representing the whole mound by weighing an equal amount of material from each sub-sample. Any ants existing in these samples were removed and added to the ant sample of the corresponding nest. One sample from the bottom of the mound (decomposed nest material, no ants observed in samples, fresh weights 36–89 g) was collected using a shovel from each mound. Litter and topsoil (to the depth of 5 cm) were collected at 15 m and 30 m distances from the mounds. These distances were selected based on the finding that colonies of red wood ants usually forage within 30 m distance from the nest (Niemelä and Laine, 1986). Four sub-samples distributed evenly at the radius (15 m or 30 m) were collected around the nests 2, 5 and 6. In case of the nests 1, 3 and 4 there were natural obstacles (e.g., pond or a large rock) preventing the collection of all four sub-samples representing the distance of 30 m. Two or three sub-samples were collected in these cases. Fresh weights of the individual litter samples varied from 1.2 g to 14 g and those of topsoil samples from 7.7 g to 110 g. The sub-samples were combined to composite samples representing each distance (15 or 30 m) around each nest by weighing equal amounts of material from each sub-sample.

### 2.3. Chemical analyses

All samples were oven-dried at 60 °C for 24 h (litter) or 48 h (ants, nest materials, soil). The dried ants were pulverized by a plastic pestle in a beaker. Separate equipment was used for each sample. Litter and nest material were mechanically ground using an analytical mill (IKA Yellowline A10). The samples of topsoil and decomposed nest material were sieved to the fraction of <2 mm. The pH of soil samples was analyzed using 1:5 soil:water volume ratio.

Chemical analyses were conducted at the laboratory of Eurofins Labtium Ltd in Kuopio. The laboratory is accredited according to FINAS T025 (EN ISO IEC 17025). All samples were digested by nitric acid in a microwave oven following the US-EPA standard 3051. Inductively coupled plasma-mass spectroscopy (Thermo Scientific iCAP Qc) was used to analyze concentrations of Ag, As, Be, Bi, Cd, Co, Cr, Cu, Mo, Ni, Sb, Se, Sn, Sr, Th, Tl, U, V and W, and inductively coupled plasma-optical emission spectroscopy (Thermo Electron iCAP 6500 Duo) to analyze those of Al, B, Ba, Ca, Fe, K, Mg, Mn, Na, P, Pb, S, Ti and Zn. The analyses included blanks and duplicate analyses were carried out systematically for 5% of the samples. Tomato leaves (SRM 1573a) and lake sediment (NW-WQB-1) were used as certified reference materials.

### 2.4. Data handling and statistical analyses

For each nest, representative concentrations in soil and litter were calculated as the arithmetic mean of concentrations at the two sampling distances (15 and 30 m). Concentration ratios for different elements  $i$  were calculated using the equation  $CR = C_{i,ant}/C_{i,soil}$ , where  $C_{i,ant}$  is the concentration of an element  $i$  in ants ( $\text{mg kg}^{-1}$  dry weight) and  $C_{i,soil}$  is the concentration of an element  $i$  in soil ( $\text{mg kg}^{-1}$  dry weight).

The transfer of elements into ants via aphids was considered using spruce needles as a proxy. The most important “aphid farms” of red wood ants are in spruces. The aphids feed on phloem sap of the spruces and elements transfer to ants directly via aphids or via honeydew, which are the main food sources of ants. Element concentrations in spruce needles were estimated based on CRs determined in our previous study

conducted at a site (in Nilsjö, Eastern Finland) where small-scale U ore prospecting have been carried out (Roivainen et al., 2011a, 2011b). Soil element concentrations were quite similar compared to those observed in this study and thus CRs observed in Nilsjö ( $N = 26$ ) should be good estimates of CRs at the current study site. However, soil U concentration was clearly elevated at the current study site when compared to Nilsjö. Therefore, only CRs representing highest soil concentrations ( $>6 \text{ mg kg}^{-1}$ ;  $N = 5$ ) observed in Nilsjö were used to estimate needle concentrations. Geometric means of observed CRs for each element were used for calculations (values shown in Table S1). The soil element concentration around each of the six mounds was multiplied by the CR of that element and the geometric mean of these six values was calculated for every element. The estimated element concentrations in spruce needles were used to evaluate the importance of transfer via aphids.

GraphPad Prism Version 5.03 (GraphPad Software, La Jolla, CA, USA) was used for statistical analyses. Relationships between ant and soil concentrations of elements were investigated using Pearson correlation coefficients and scatterplots showing soil element concentration on x-axis and ant element concentration on y-axis. A horizontal line ( $y = a$ ) and a line through origin ( $y = ax$ ) were fitted to each scatterplot. Absolute sums of squares were used as a measure of the goodness of fit.

## 3. Results

### 3.1. Element concentrations in different compartments

The average dry weight content was 36% (range 34–37%) for ants, 91% (83–94%) for nest material, 88% (82–94%) for decomposed nest material and 84% (77–88%) for litter. The dry weight content of the soil samples was more variable (average 63%, range 42–85%). Soil pH varied from 4.05 to 5.55 (average 4.60). The measured concentrations of elements in ants, nest material, decomposed nest material, litter, and soil as well as the estimated concentrations in spruce needles are shown in Table 1. The geometric standard deviations (GSDs) showed that the variation of element concentrations between nests was in general smaller in ants than in nest material. Comparison of GSDs also showed that soil element concentrations tended to vary more than litter concentrations between nests. Regarding to element concentrations, the variation was greatest in U concentrations.

In general, the element profile of ants resembled that of spruce needles more than those of soil, nest material or litter (Fig. 2). Elements that were clearly accumulated in ants in comparison to any other compartment were Cd and Zn in addition to the main nutrients K, P and S. The concentrations of Cu and Mo were elevated in ants when compared to the estimated concentrations in spruce needles. Ba and Sr were quite evenly distributed between these two compartments while it seemed that their chemical analogue Ca was not transferred to ants that efficiently. No clear accumulation of Mg and Mn in any studied compartment was observed. U concentration in ants was slightly elevated when compared to spruce needles but clearly lower than in other compartments. For the other elements studied (for which concentrations above detection limits were observed), transfer from soil to spruce and ants seemed to be low and no clear differences between spruce and ant concentrations were observed.

Litter and nest material concentrations of most elements corresponded to each other (Fig. 3). However, the concentrations of Cd and Zn (which accumulated in ants) tended to be lower in nest material than in litter. On the contrary, concentrations of some elements that showed low transfer to ants or spruce needles (e.g. Al, Fe, Ti, V) were elevated in nest material when compared to litter. The concentrations of several elements differed between undecomposed nest material and decomposed nest material collected from the bottom of the mound (Fig. 3). Ag, Al, As, Cu, Fe, Mg, Th, Ti, Tl, U and V were accumulated in decomposed nest material. Most of these elements are not needed as nutrients by plants or other organisms but there are few exceptions like Cu, Mg and Fe. The elements for which no accumulation in decomposed nest

**Table 1**

Geometric mean (GM) and geometric standard deviation (GSD) of element concentrations ( $\text{mg kg}^{-1}$  dry weight) in ants, nest material, decomposed nest material, litter and soil collected from six mounds in the vicinity of a former uranium mine. Element concentrations in spruce needles in the vicinity of nest mounds were estimated based on concentration ratios observed in Nilsjö, Eastern Finland (see [Supplementary Table 1](#)). The value shown is the GM and GSD of calculations for each mound ( $n = 6$ ). Ranges are shown in cases where some of the results were below detection limits.

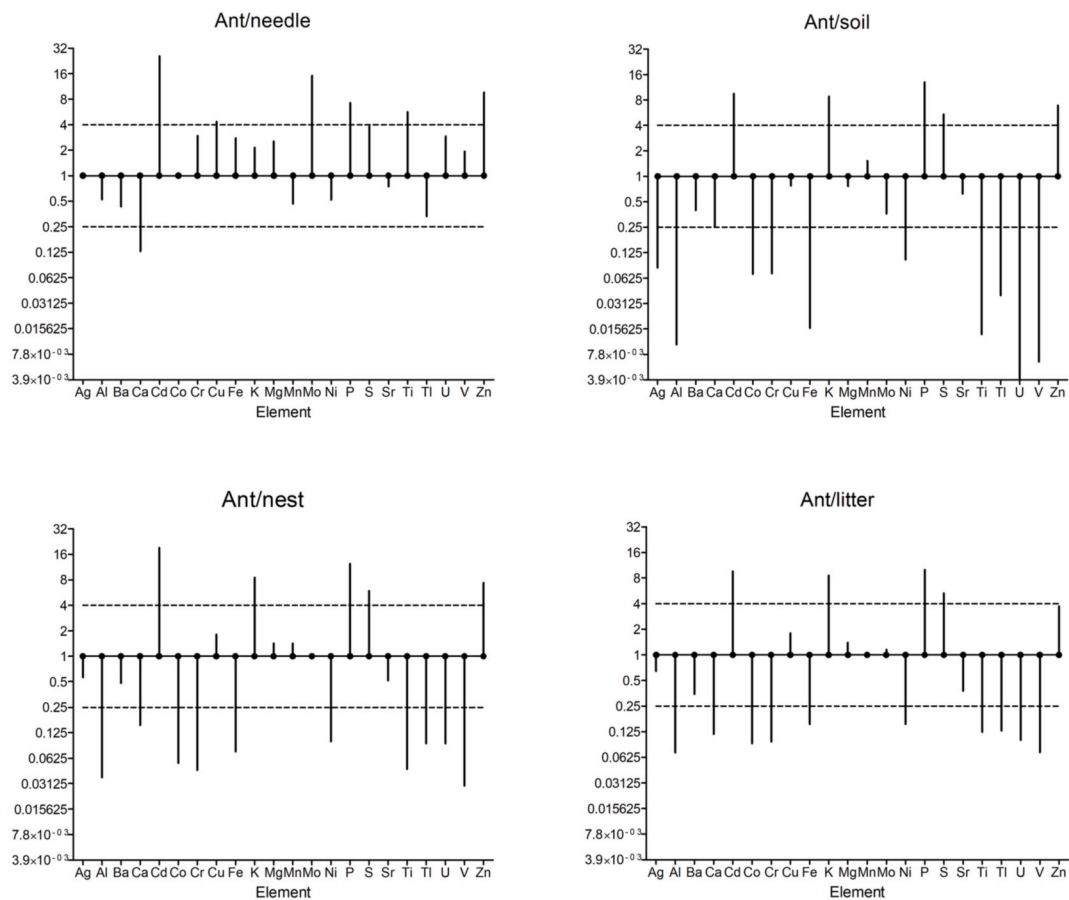
Element	Ant	Nest	Decomposed	Litter	Soil	Needle
	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)
Ag	<0.01–0.02	0.0178 (1.33)	0.0377 (1.62)	0.0156 (1.46)	0.121 (2.43)	0.0103 (2.25)
Al	38.2 (1.45)	1030 (1.68)	5290 (1.69)	536 (1.25)	3730 (1.81)	73.0 (1.72)
As	<0.05–0.06	0.299 (1.94)	1.23 (2.18)	0.151 (1.44)	1.58 (1.47)	n.c. <sup>a</sup>
B	<5–6	<5–6	<5	<5–7	<5	n.c. <sup>b</sup>
Ba	29.5 (1.34)	61.7 (1.63)	45.8 (1.58)	85.7 (1.13)	74.0 (1.54)	68.7 (1.48)
Be	<0.05	<0.05–0.1	0.0844 (2.77)	<0.05	0.0772 (1.95)	n.c. <sup>a</sup>
Bi	<0.2	<0.2	<0.2–0.21	<0.2	<0.2–0.61	n.c. <sup>a</sup>
Ca	1120 (1.18)	7310 (1.41)	2840 (1.68)	9550 (1.23)	4330 (1.76)	8750 (1.68)
Cd	3.07 (1.11)	0.161 (1.21)	0.124 (1.36)	0.319 (1.29)	0.323 (1.80)	0.119 (1.71)
Co	0.300 (2.05)	5.50 (2.31)	3.21 (2.15)	3.31 (1.63)	4.29 (2.32)	0.292 (2.15)
Cr	0.736 (1.04)	16.4 (2.31)	12.4 (2.08)	7.69 (1.60)	10.4 (2.04)	0.248 (1.92)
Cu	13.4 (1.17)	7.42 (1.51)	17.3 (1.69)	7.45 (1.26)	17.2 (1.48)	3.09 (1.42)
Fe	99.4 (1.11)	1330 (1.86)	7240 (1.98)	646 (1.57)	6220 (1.75)	35.7 (1.67)
K	9200 (1.08)	1080 (1.33)	1310 (1.38)	1070 (1.14)	1040 (1.32)	4260 (1.29)
Mg	1310 (1.07)	927 (1.35)	2150 (2.00)	943 (1.20)	1710 (2.12)	514 (1.99)
Mn	887 (1.10)	624 (1.32)	342 (1.85)	909 (1.27)	579 (2.18)	1900 (2.04)
Mo	0.465 (1.51)	0.500 (1.76)	0.475 (3.06)	0.403 (1.78)	1.28 (2.00)	0.0307 (1.88)
Na	2810 (1.07)	<50	<50–57	<50	<50–54	n.c. <sup>b</sup>
Ni	1.11 (1.33)	11.2 (1.90)	9.66 (2.07)	7.21 (1.28)	10.7 (1.73)	2.14 (1.65)
P	8790 (1.09)	711 (1.17)	554 (1.27)	878 (1.15)	675 (1.12)	1210 (1.11)
Pb	<5	<5–5	7.82 (2.19)	<5	22.5 (2.67)	0.332 (2.45)
S	4870 (1.07)	819 (1.23)	437 (1.76)	924 (1.14)	902 (1.75)	1270 (1.67)
Sb	<0.02	0.0377 (1.22)	0.0222 (1.99)	0.0362 (1.20)	0.117 (2.29)	n.c. <sup>a</sup>
Se	<0.5–0.62	<0.5	<0.5	<0.5	<0.5–1.12	n.c. <sup>a</sup>
Sn	<0.1	<0.1	<0.1–0.12	<0.1–0.14	0.201 (1.68)	n.c. <sup>a</sup>
Sr	11.5 (1.38)	22.2 (1.68)	27.7 (1.66)	30.6 (1.37)	18.6 (1.75)	15.4 (1.67)
Th	<0.02	0.223 (1.56)	1.59 (1.72)	0.0923 (1.89)	1.54 (1.95)	n.c. <sup>a</sup>
Ti	2.88 (1.61)	62.0 (1.57)	330 (1.22)	23.1 (1.54)	214 (1.46)	0.510 (1.41)
Tl	<0.01	0.0507 (1.51)	0.0847 (1.55)	0.0390 (1.46)	0.128 (1.44)	0.0150 (1.39)
U	0.123 (2.89)	1.32 (4.15)	4.14 (6.80)	1.24 (1.80)	30.4 (3.39)	0.0423 (3.04)
V	<0.1–0.17	3.41 (1.38)	18.6 (1.50)	1.40 (1.44)	15.7 (1.63)	0.0520 (1.56)
W	<0.1	13.8 (2.72)	<0.1	6.49 (2.50)	0.144 (2.53)	n.c. <sup>a</sup>
Zn	416 (1.23)	56.5 (1.21)	34.2 (1.46)	111 (1.31)	60.0 (1.62)	43.2 (1.55)

n.c. = not calculated.

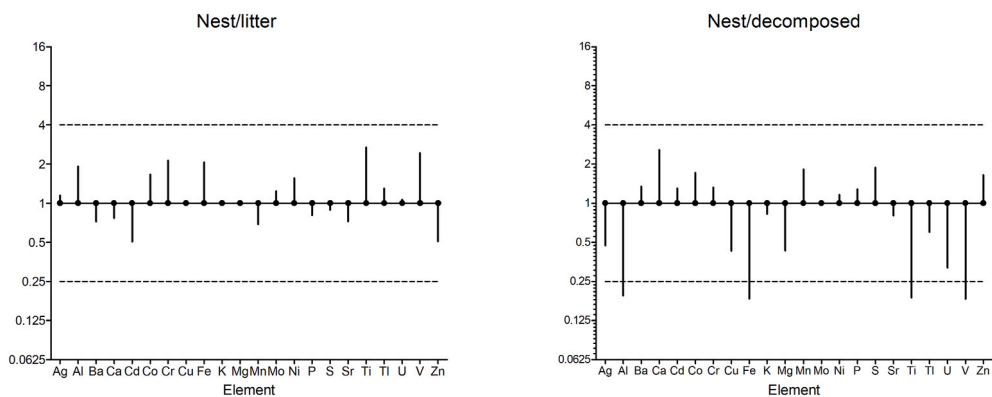
<sup>a</sup> Information on spruce needle CRs in Nilsjö not available.

<sup>b</sup> Element concentration in soil below detection limit.





**Fig. 2.** Comparison of element concentration profiles observed in ants to those in potential sources of elements. The ant/needle, ant/soil, ant/nest material and ant/litter ratios are shown for each element. The dashed lines indicating 4-fold greater or lower concentration in ants than in the potential source being investigated were added to the figure to help visualization.



**Fig. 3.** The ratio of element concentrations between nest material and litter and nest material and decomposed nest material. The dashed lines indicating 4-fold greater or lower concentration in nest material than in the other compartment being investigated were added to the figure to help visualization.

material was observed (Ca, Mn, S, Zn) were mostly important plant nutrients. The elements that were quite evenly distributed between the two compartments studied (Ba, Cd, Co, Cr, K, Mo, Ni, P, Sr) include important nutrients as well as elements considered nonessential to plants and other organisms.

### 3.2. Concentration ratios

Although direct contact with soil is not likely to be an important transfer route for all elements, CRs between ants and soil were

calculated (Table 2). Current radioecological models commonly utilize organism-to-soil CRs; soil is in any case the primary source of elements, even if their transfer route into ants includes uptake from soil by spruces (and other plants), and aphids (and other prey animals) as intermediate steps.

The use of CRs includes the assumption that element concentrations in organisms are linearly related to medium concentrations. Pearson correlation coefficients between ant and soil concentrations were not statistically significant for any element studied, and many were negative (Table S2). The scatterplots shown in Fig. 4 also indicated that most of

**Table 2**  
Geometric means (GM) and geometric standard deviations (GSD) of concentration ratios of elements (mg kg<sup>-1</sup> dry weight/mg kg<sup>-1</sup> dry weight) between ants and soil.

Element	GM (GSD)
Al	0.0103 (2.15)
Ba	0.398 (1.83)
Ca	0.258 (1.70)
Cd	9.49 (1.84)
Co	0.0699 (1.69)
Cr	0.0706 (2.10)
Cu	0.776 (1.35)
Fe	0.0160 (1.71)
K	8.84 (1.27)
Mg	0.764 (2.02)
Mn	1.53 (2.21)
Mo	0.364 (1.97)
Ni	0.104 (1.69)
P	13.0 (1.18)
S	5.39 (1.82)
Sr	0.618 (1.78)
Ti	0.0135 (1.70)
U	0.00406 (3.44)
Zn	6.94 (1.66)

the data does not support the linearity assumption. Instead of linear increase along with increasing soil concentrations, the ant element concentrations tended to be relatively constant. This observation was supported by the comparison of the goodness of fit between a horizontal line ( $y = a$ ) and a line through origin ( $y = ax$ ) using absolute sums of squares as a measure (Table 3).

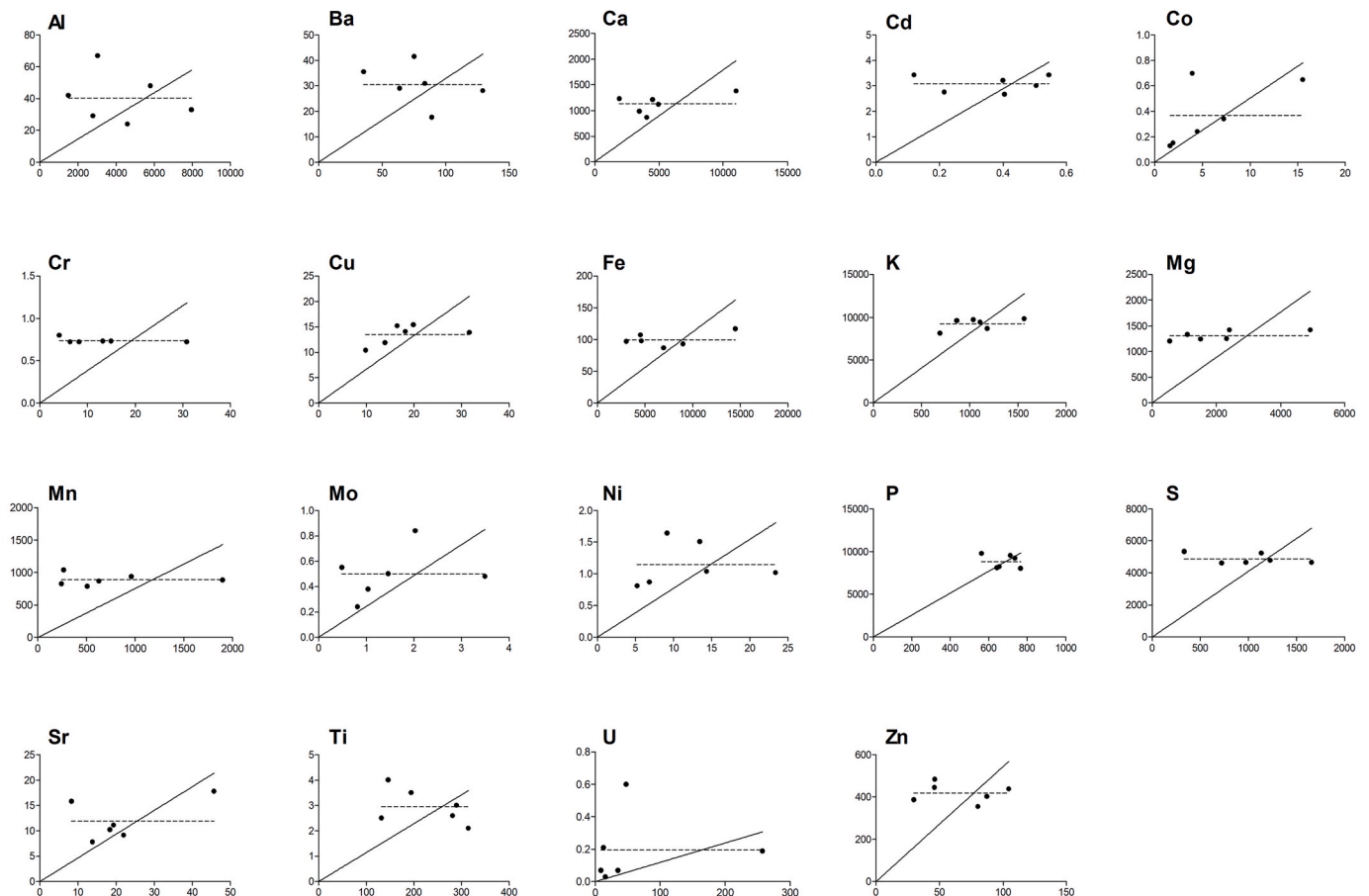
#### 4. Discussion

In this study, element concentrations in red wood ants, their nest mounds and surrounding soil were analyzed at a former uranium mine. Additional information was obtained by estimating element concentrations in spruce needles using CRs observed at a forest of similar type

**Table 3**

Absolute sum of squares of nonlinear regression for horizontal line ( $y = a$ ) or line through origin ( $y = ax$ ) fitted to scatterplots showing element concentrations in ants and soil.

Element	$y = a$	$y = ax$
Al	1220	380
Ba	322	1260
Ca	171,000	$1.50 \times 10^6$
Cd	0.540	8.87
Co	0.311	0.275
Cr	0.00493	1.10
Cu	19.2	99.3
Fe	569	11,500
K	$2.27 \times 10^6$	$2.38 \times 10^7$
Mg	45,200	$2.71 \times 10^6$
Mn	39,800	$1.78 \times 10^6$
Mo	0.201	0.481
Ni	0.593	2.00
P	$3.12 \times 10^6$	$1.01 \times 10^7$
S	502,000	$2.38 \times 10^7$
Sr	78.0	165
Ti	2.46	10.8
U	0.223	0.351
Zn	10,500	171,000



**Fig. 4.** Concentration in ants (mg kg<sup>-1</sup>, on y axes) as a function of concentration in soil (mg kg<sup>-1</sup>, on x axes) for the elements studied. Line through origin ( $y = ax$ , black line) and horizontal line ( $y = a$ , dashed line) are fitted to the data.

(Roivainen et al., 2011a, 2011b). The observed concentrations can be discussed from the point of view of element cycling at the site. Elements from soil are transferred to spruce needles via root uptake and possibly by other routes (e.g., deposition of dust from soil). Aphids living on spruces mediate the transfer of elements further to ants. Elements are released back to the soil when fallen spruce needles (main constituents of litter and nest material) decompose. The study was carried out at a boreal forest site, where cycling of elements can be limited because the cold soil temperatures reduce rates of organic matter decomposition (Bonan and Shugart, 1989; Yuan and Chen, 2010).

Tuovinen et al. (2016a, 2019) have recently investigated potential releases of radionuclides and metals from waste rock and tailings at the study area by taking water, sediment, and soil samples. Their results indicated on-going leaching and accumulation of  $^{226}\text{Ra}$  from the waste-rock pile and possibly tailings (Tuovinen et al., 2016a). According to the study of Tuovinen et al. (2016a)  $^{238}\text{U}$  activity concentrations in soil were above the average concentrations of Finnish soil but typical to the soil in the studied region. The increasing trend of As, Cd, Co, Cu and Fe concentrations in pond sediments gave some indication of long-term leaching but concentrations of these metals in soil were at typical environmental levels in Finland (Tuovinen et al., 2019). Our results are in agreement with those of Tuovinen et al. (2016a, 2019) as U was the only element for which we observed elevated concentrations in comparison to background values in Finnish soils.

The geometric mean of U concentrations in ants was  $0.123 \text{ mg kg}^{-1}$ , which corresponds to the activity concentration of  $1.52 \text{ Bq kg}^{-1}$  of  $^{238}\text{U}$  ( $1 \text{ mg U} = 12.34 \text{ Bq } ^{238}\text{U}$ ). There are few previous studies on U concentrations in ants. Haavisto et al. (2018) measured concentrations of several elements in red wood ants collected at two locations in southwestern Finland but they had only one sample of adult ants per site. Concentrations of U in ants collected by Haavisto et al. (2018) were  $0.0995 \text{ mg kg}^{-1}$  and  $0.00259 \text{ mg kg}^{-1}$ . The greater value corresponds to values measured in our study. Medley et al. (2017) investigated weaver ants (*Oecophylla smaragdina*) in tropical Northern Australia at a uranium mine and reference areas. Dragović et al. (2010) investigated genera *Lasius* and *Formica* in Serbia. In both studies  $^{238}\text{U}$  activity concentrations were close to those observed in our study but sample sizes were limited also in these studies.

Previous studies of element concentrations in red wood ants in Finnish forest have mainly focused on certain elements (Al, As, Cd, Cu, Ni, Pb and Zn) and areas around metal industries in western Finland (e.g., Eeva et al., 2004; Skaldina et al., 2018). The concentrations measured in our study were mostly in agreement with those observed in reference areas used by Eeva et al. (2004) and Skaldina et al. (2018). Skaldina et al. (2018) also measured Sr concentrations and the values obtained for ants in the industrial area were closer to our values than those obtained at the reference area although the difference to the reference area was rather small. Based on these comparisons, it seems that element concentrations in ants at our study site resemble those measured in typical Finnish forests.

Elements such as Al, Fe, Ti and V showed higher concentrations in nest material (especially in the decomposed fraction) than in litter, although litter is an important constituent of nests. These elements may have been transferred to the nests from another sources, and it is possible that the behavior of ants has affected this accumulation. Ants may have collected food from further away from the nest and after feeding excreted these non-essential elements to the nests (Frouz et al., 2005). Another potential explanation is that elements accumulated in deeper soil layers are transferred to the nest when ants mix the soil through excavation (Frouz et al., 2005).

Elements that were accumulated in ants in comparison to other compartments studied were mostly important nutrients (K, P, S, Zn), Cd being an exception. The accumulation of Cd and Zn in ants has been observed also in other studies (Starý and Kubizňáková, 1987; Rabitsch, 1995; Grześ, 2010b; Skaldina et al., 2018). It is known that honeydew can be a significant source of metals, e.g., Cd (Starý and Kubizňáková,

1987) and it is therefore likely that transfer via honeydew has been important also at our study site although it could not be investigated directly. Cd and Zn have radioactive isotopes but are not considered the most important from the point of view of radiation protection. Among elements studied, those that are considered more important in this sense (Ag, Mo, Ni, Pb, Se, Sn, Sr, Th, U) did not seem to accumulate in ants. However, Mo concentrations in ants were elevated when compared to the estimated concentration in spruce needles.

In their study conducted in Serbia, Dragović et al. (2010) reported ant/soil CRs of  $^{238}\text{U}$  ranging from 0.05 to 0.07 for three ant samples representing genus *Lasius* and value of 0.08 for a sample representing genus *Formica*. These values are one order higher than CRs of U calculated in our study. Individual species are not handled separately in databases containing transfer parameters to be used in radioecological modelling, but larger groups of reference organisms typical for major environments are considered (Coppelstone et al., 2013; IAEA, 2014; ICRP, 2014; Brown et al., 2016). Ants can be considered to belong to the group “Arthropod” in IAEA reference organisms (IAEA, 2014). Concentration ratios are collected to the Wildlife Transfer Parameter Database (Coppelstone et al., 2013). The geometric mean of arthropod/soil CR value for U in this database is 0.0076 which is of same order as the fresh weight-converted value observed in our study. The CR values for Ca, Cd, Cu, Fe, Mo, Sr, and Zn observed in our study also corresponded to the values collected to the database. For the other elements studied, the values observed in our study tended to be greater than the values reported in the database. In these cases, the use of the values existing in the database may lead to underestimation of element uptake of ants.

Studies on forest plants and earthworms have showed that non-linear models perform better than conventional linear CR in describing transfer of many elements (Tuovinen et al., 2011, 2016b; 2016c). The analyses in the present study were affected by the small sample size and limited range of soil concentrations in case of many elements. However, a general trend was observed suggesting that ant element concentrations were relatively constant regardless of the soil concentration. Horizontal lines fitted the data for most elements better than lines through origin. This might be related to the regulation of metal concentrations by ants (Grześ, 2010a, 2010b). There is some contradiction between our results and a previous study conducted in Italy. Frizzi et al. (2017) reported that concentrations of Cd, Co, Cr and Ni in ants (*Crematogaster scutellaris*) were significantly related to those in soil while concentrations of Cu and Zn were not. One explanation for this could be differences in soil chemistry and geology as these factors have an impact on bioavailability of elements. Another point is that concentrations of most metals were elevated and represented a wider range in the study of Frizzi et al. (2017) than in our study. Previous studies focusing on plants and soil invertebrates have suggested that non-linearity of transfer is especially important at low concentrations in soil (Tuovinen et al., 2011, 2016b). Based on our results this seems to be true also for ants. Tuovinen et al. (2016c) introduced a non-linear approach to describe soil-to-plant uptake of radionuclides. This was based on the observation that plant total element concentrations were constant. Our results show that a similar approach might estimate the uptake of radionuclides into ants better than conventional CRs but should be validated with larger datasets.

The role of food sources on accumulation of elements in ants could be considered in more detail in further studies. Here we used estimated element concentrations in spruce needles (host trees of “aphid farms”) as a proxy of this transfer route. It seemed that the element profile of ants was similar to that of spruce needles, but it is hard to draw definite conclusions on transfer of elements through food. Future studies could include sampling of spruce needles and/or aphids and honeydew. Sampling of different plants could also help to get a more detailed picture of the role of ants in transfer of elements/radionuclides in forest ecosystems. Although information obtained using stable elements is relevant also for radionuclides, measurements of radionuclides would be useful. The physical size of ants however is challenging as analyses of stable



element concentrations (Medley et al., 2017). More detailed information on biogeochemical factors at the study site as well as measuring potentially bioavailable soil element concentrations instead of total concentrations would have improved the study.

## 5. Conclusions

Ant U concentrations were low in comparison to soil or nest material. Elements that accumulated in ants were Cd, K, P, S and Zn as observed also in many previous studies. Comparison of ant/soil CR values at the study site to arthropod/soil CR values available in commonly utilized international database showed that the values were comparable for Ca, Cd, Cu, Fe, Mo, Sr, U and Zn. For the other elements studied, the use of database values is likely to underestimate the soil-to-ant transfer in boreal forest sites. Ant element concentrations tended to be constant regardless of the soil concentrations. Non-linear transfer models might describe the soil-to-ant transfer better than conventional CRs and increase the accuracy of radioecological models. Further studies with wider range of soil concentrations are needed to validate these observations.

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## CRedit authorship contribution statement

**Päivi Roivainen:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Project administration. **Saara-Maria Muurinen:** Formal analysis, Investigation, Writing – review & editing. **Jouni Sorvari:** Methodology, Writing – review & editing. **Jukka Juutilainen:** Conceptualization, Methodology, Writing – review & editing. **Jonne Naarala:** Conceptualization, Writing – review & editing. **Sisko Salomaa:** Conceptualization, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2022.119231>.

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