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# Effects of heavy metal pollution on red wood ant (*Formica* s. str.) populations

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**"Capsule":** *Five species of red wood ants vary in their sensitivity to heavy metal pollution but all of them had smaller colonies in a polluted area.*

## Abstract

We studied the species composition, mound population densities, relative abundance and colony sizes of red wood ants along a well known air pollution gradient of a copper smelter in Southwest Finland. The dominant species, *Formica aquilonia*, was further studied for heavy metal (Al, Cu, Cd, Ni, Zn, As, Pb, Hg) levels and morphological characters (body mass, head width, labial gland disease) of workers. We found five species belonging to *Formica* s. str., and two of them showed changes in their relative abundance, which could not be explained by natural habitat differences. Nest mound volumes were 34% smaller in the polluted area, suggesting that smaller colonies can be maintained there. The heavy metal levels in *F. aquilonia* workers were higher in the polluted area for all metals, except Hg. The largest relative differences between the study areas (polluted/unpolluted) were found for As (4.1), Ni (2.4), Cu (2.1) and Pb (1.8). Morphological characters of workers were not related to the heavy metal levels. Our data showed that red wood ants can tolerate relatively high amounts of heavy metals and maintain reproducing colonies even in a heavily polluted area, but on the basis of smaller colony sizes, pollution stress may also cause trade-offs in reproduction.

*Keywords:* Air pollution; Ants; Environmental stress; Formicidae; Heavy metals; Hymenoptera; Labial gland disease

## 1. Introduction

Ants are known to easily accumulate heavy metals and they are considered to be good indicators of pollution because they are high in the food chain and the members of their colonies are less mobile than insects of many other groups (Bengtsson and Rundgren, 1984; Hoffmann et al., 2000; Migula and Glowacka, 1996; Nuorteva, 1999; Rabitsch, 1995; Stary and Kubizňáková, 1987). Due to their perennial stationary colonies, it is also possible to measure temporal changes in concentrations, morphological traits or offspring production and to get estimates on colony survival.

Although some ant species are found to be relatively resistant to pollutants, the populations of red wood ants (*Formica* spp.) have decreased in many places where forests are affected by atmospheric pollution (Katayev et al., 1983; Koricheva et al., 1995; Podkowka, 1984; Stary and Kubizňáková, 1987). However, the connection between declining populations and levels of pollutants has not been convincingly showed so far. We think that there are two reasons for this. First, pollutant levels are often analysed separately from the information on natural habitat characteristics, making interpretation of field data difficult. Secondly, many environmental studies are made at a group level (like

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*Formica rufa* group), consisting of variable number of closely related species. Because different species have different life strategies (Rosengren et al., 1993) and habitat requirements, they may respond differently to pollution. Therefore, we think that separate analyses for each species are essential.

In this study, we inspected the species composition, mound population densities, relative mound abundance and colony size of red wood ants along a well known air pollution gradient of a copper smelter in Southwest Finland. Several habitat variables concerning the predominant forest trees (the main feeding substrate for red wood ants) were measured to separate pollution effects from natural habitat variation. Air pollution is supposed to pose an extra stress for insects that can cause trade-offs, e.g., in reproduction, growth or resistance to infectious diseases (Pimentel, 1994). We used nest mound volume as an index of colony size, which is dependent on the ability of a colony to reproduce. The most common species, *Formica aquilonia* Yarrow, was further studied for its heavy metal levels and morphological characters (body mass, head width) of workers. To find out whether pollution stress makes ants more vulnerable to infections, we inspected workers for an infectious labial gland disease (Elton, 1991).

## 2. Materials and methods

### 2.1. Study area and data collection

We collected the data in summer 2002, in the surroundings of a copper smelter in the town Harjavalta (61°20′ N, 22°10′ E), SW Finland. Sulphuric oxides and heavy metals (especially Cu, Zn, Ni, Pb and As) are common pollutants in the area (Jussila and Jormalainen, 1991; Kubin, 1990). Elevated heavy metal concentrations occur in the polluted area due to current and long-term deposition, and metal contents decrease exponentially with increasing distance to the smelter approaching background levels at sites further than 5 km from the smelter (Eeva and Lehikoinen, 1996; Jussila et al., 1991; Koricheva and Haukioja, 1995). The forests in the area are dominated by Scotch pine *Pinus sylvestris* L., which forms mixed stands with Norway spruce *Picea abies* L. and birches (*Betula* spp.). In the field layer, dwarf shrubs (*Vaccinium vitis-idaea* L. and *V. myrtillus* L.) dominate. At the sites closest to the factory complex, ground layer vegetation is patchy and poorly developed due to the long-term effect of pollution (Salemaa and Vanha-Majamaa, 1993).

In June, we searched for all mound building *Formica* (s. str.) colonies at 14 study plots ($\bar{X} = 7.9 \pm 1.22$ ha; total area 111 ha), which were at the distances of 0.9–11.2 km from the pollution source to three main directions (SW, NW and SE). The study plots were not isolated forest stands but parts from larger forest areas. Altogether 113 colonies were found. From two nests of the same species less than 20 m from each other, we selected only the one which was found first because of the high probability that they belonged to the same colony. On this basis, four nests were discarded from all analyses. Nest mounds were numbered and their locations were determined with GPS. The mean distance of a nest mound to a neighbouring nest was 70 m (±8.7, $n = 109$). For calculation of nest mound volumes, the mound height was measured at two opposite sides (south and north) and basal diameter was cross-measured (south–north and east–west). The means of the two measures were used to calculate the mound volume (volume of paraboloid; $V$ [l] $= 0.5 \times$ basal area [dm$^2$] $\times$ height [dm]), which was used as an index of colony size (Wuorenrinne, 1989).

Special attention was paid in selecting study plots so that they would represent a similar forest type, i.e. relatively barren pine dominated forests typical of the study area. To account for the remaining variation in tree species composition, we estimated timber volumes for dominant tree species (pine, spruce, birches) by using a relascope and hypsometer: volume [m$^3$ ha$^{-1}$] = basal area [m$^2$ ha$^{-1}$] $\times 0.5 \times$ tree height [m]. These tree species are the main foraging substrate for red wood ants in our study area (Rosengren and Sundström, 1991). The timber volumes of coniferous (pine + spruce) and deciduous (birches) trees were used as explaining factors in the analyses of ant species composition to separate the effects of pollution from other habitat variation (due to forestry and natural differences). Although field layer vegetation also differed among the study sites, we did not include this variable in the analyses because it was strongly affected by pollution (see above) and these differences can mainly be considered as secondary effects of pollution.

It has been shown that surface workers tend to accumulate highest concentrations of heavy metals among different red wood ant castes (Maavara et al., 1994). We collected two samples of worker ants from the surface of each nest mound in Eppendorf tubes: a sample for species identification and morphometric measurements and a sample for heavy metal analyses. Samples for species identification were preserved in ethanol and samples for heavy metal analyses were kept frozen until dried in laboratory. After the identification of species, ten individuals from 37 *F. aquilonia* colonies were measured in laboratory for their body mass and head width ($n = 370$ individuals). Head width has shown to be a good measure of body size in red wood ants (Rabitsch, 1995). They were further inspected under a light microscope for an infectious labial gland disease, which is expressed by swelling of the labial gland (Elton, 1991). Three individuals were lost before taking all the measurements and they were omitted from the subsequent analyses. Also the sole ant with labial gland syndrome was omitted from the analyses of morphological traits.

### 2.2. Heavy metal analyses

The most common species, *Formica aquilonia*, was selected for heavy metal analyses. Samples ($n = 37$) were dried at 50 °C for 48 h and analysed for contents of Al, Cu, Cd, Ni, Zn, As, Pb and Hg. The samples were accurately weighed in the range 0.15–0.20 g. Two millilitres of supra-pure $HNO_3$ acid and 0.5 ml of $H_2O_2$ were added to the samples into Teflon bombs for digestion with a microwave system (Milestone High Performance Microwave Digestion Unit MLS 1200 mega). After the digestion, the samples were diluted to 50 ml with de-ionized water (Elgastat Maxima). The determination of concentration of the analyte elements was done with inductively coupled plasma mass spectrometer (ICP-MS) Elan 6100 DRC+ from PerkinElmer–Sciex (Montaser, 1998). The detection limits for most of the elements are around ppt ($ng\,l^{-1}$) level and below. The calibration of the instrument has been done with certified solution (Claritas PPT, Multi element solution 2A) from Spex Certiprep.

### 2.3. Statistics

For the analyses of mound population density, relative mound abundance, mound volumes and heavy metal concentrations, the data were divided into two parts: sites closer than 2 km from the smelter were considered to be in a polluted area and sites more than 4 km from the smelter were considered to be in an unpolluted area. Estimates on mound population densities (nests per ha) were calculated for each study plot using nearest neighbour distance method (Krebs, 1989), and the means were compared between two study areas with an ANOVA. Relative mound abundances in the two areas were compared with chi-square tests. Nest mound volumes were log-transformed and compared among species and between the study areas with a two-way ANOVA by using species-specific site averages as replicates.

The simultaneous effects of habitat and distance to pollution source were further studied by using generalized linear models, where distance (km) to the pollution source and timber volumes ($m^3\,ha^{-1}$) of coniferous and deciduous trees were used as dependent factors. The occurrence of a species (0 = no, 1 = yes) was used as a binomial independent factor. Timber volumes of coniferous and deciduous trees were not significantly correlated ($r = -0.18$, $P = 0.06$). In these models, binary distribution and logit link function were used.

Heavy metal concentrations were log-transformed before the analyses to make distributions normal. One exceptionally high value ($198\,\mu g\,g^{-1}$) for Al was discarded from the analyses. Cluster analysis was used to group the heavy metal data on the basis of similarity in concentration variation. The data used in the cluster analysis were Pearson correlations among individual metals and the analysis was based on unweighted pair-group method using arithmetic means.

Differences in body mass, head width (size) and residual mass (residuals from linear regression where body mass is explained by head width) between the two study areas were tested with nested mixed-model ANOVAs, where mound (a random factor) was nested within study areas. To study the association of these three variables with habitat characteristics and pollutant levels, we correlated mound means with timber volumes and heavy metal concentrations.

For each parametric test, a normality of residuals was tested with Shapiro–Wilk test. All the analyses were done in SAS statistical system for Windows (SAS Institute, 2001).

## 3. Results

### 3.1. Species composition, mound densities and relative abundances

We found five species of *Formica* s. str. in the study area occurring both in the polluted and unpolluted environments (Table 1). There were no differences in total nest mound densities between the two study areas, as calculated using nearest neighbour distances (polluted area: $\bar{X} = 0.94 \pm 0.40$ nests/ha, $n = 7$ sites; unpolluted area: $\bar{X} = 0.96 \pm 0.32$ nests/ha, $n = 7$ sites; ANOVA, $F_{1,12} = 0.00$, $P = 0.98$). The mounds of the two dominant species, *F. aquilonia* and *F. polyctena* Förster, were found at equal probability in the polluted and unpolluted areas, i.e. pollution had no effect on the relative mound abundance of these species (Table 1). One species, *F. lugubris* Zetterstedt, was less abundant and two species, *F. rufa* L. and *F. pratensis* Retzius, were proportionally more abundant in the polluted area than in the unpolluted area (Table 1).

Table 1
The species and numbers of *Formica* nest mounds at two study sites

| Species | Polluted area | | Unpolluted area | | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | |
| *F. aquilonia* | 20 | 50.0 | 44 | 63.8 | 1.4 | 0.24 |
| *F. polyctena* | 8 | 20.0 | 10 | 14.5 | 0.4 | 0.53 |
| *F. lugubris* | 1 | 2.5 | 12 | 17.4 | 5.69 | 0.017 |
| *F. rufa* | 6 | 15.0 | 2 | 2.9 | 5.06 | 0.025 |
| *F. pratensis* | 5 | 12.5 | 1 | 1.5 | 5.58 | 0.018 |
| Total | 40 | 100 | 69 | 100 | | |

Chi-square tests for differences in nest frequencies between study sites ($n = 109$ nests).

Table 2
The effects of distance (km) to the pollution source and timber volumes ($m^3 ha^{-1}$) of coniferous and deciduous trees on the relative mound abundance of five *Formica* species along the air pollution gradient

| Source of variation | *F. aquilonia* | | *F. polyctena* | | *F. lugubris* | | *F. rufa* | | *F. pratensis* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| Distance | 0.05 | 0.82 | 0.10 | 0.75 | 11.3 | 0.0008 | 5.48 | 0.019 | 0.00 | 0.98 |
| Vol. coniferous | 25.8 | <0.0001 | 4.25 | 0.039 | 8.22 | 0.0042 | 0.05 | 0.82 | 14.7 | 0.0001 |
| Vol. deciduous | 0.12 | 0.73 | 0.11 | 0.74 | 2.25 | 0.13 | 0.04 | 0.84 | 3.52 | 0.061 |

Generalized linear models ($n$ = 109 nests).

The occurrence of four species was related to the timber volume of coniferous trees around the colony. The relative abundance of *F. aquilonia* increased and the relative abundance of *F. polyctena*, *F. lugubris* and *F. pratensis* decreased with increasing timber volume of conifers (Table 2; Fig. 1a). The occurrence of *F. rufa* showed no association with timber volume of conifers and none of the species showed significant associations with timber volume of deciduous trees (Table 2). After adding the habitat variables into the models, the effect of distance to the pollution source was significant in two species: *F. lugubris* was less abundant and *F. rufa* was proportionally more abundant in the polluted area than in the unpolluted area (Table 2; Fig. 1b).

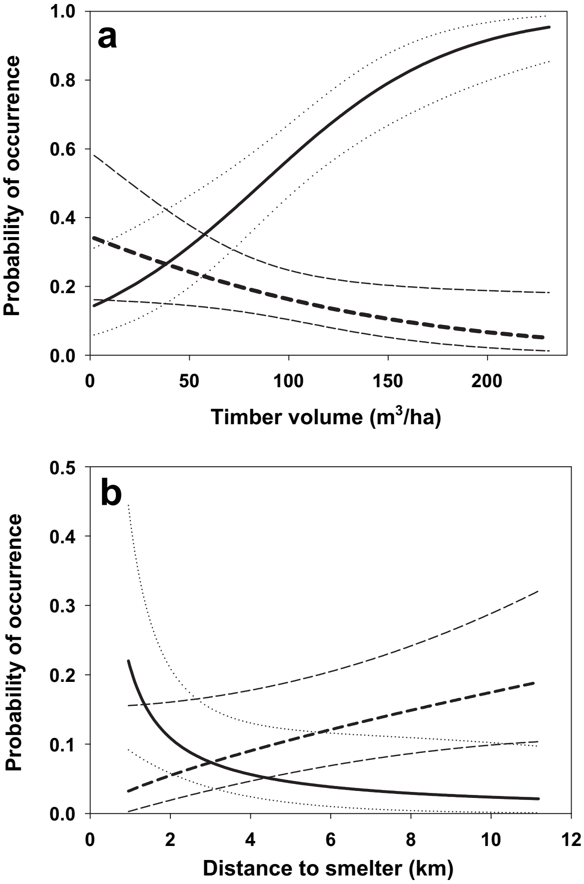

Fig. 1. The probabilities (±95% confidence limits) of a nest mound to belong to a certain *Formica* species in relation to (a) the timber volume of coniferous trees around the nest (*Formica aquilonia* — and *Formica polyctena* ----) and (b) the distance to the smelter (*Formica rufa* — and *Formica lugubris* ----). Generalized linear models ($n$ = 109 nests).

### 3.2. Mound volumes

Nest mound volumes were 34% smaller in the polluted area ($\bar{X} = 157 \pm 29.3$ l, $n = 40$) than in the unpolluted area ($\bar{X} = 238 \pm 33.1$ l, $n = 69$; ANOVA for species-specific site averages: $F_{1,26} = 5.31$, $P = 0.030$). The nest mound volumes did not significantly differ among the species (ANOVA: $F_{4,26} = 1.10$, $P = 0.38$) and there was no interaction between species and study area (ANOVA: $F_{4,26} = 0.78$, $P = 0.54$), suggesting that the change was similar in all species. However, a small number of nests of *F. lugubris*, *F. rufa* and *F. pratensis* weaken the power of the test to detect significant interactions among species.

### 3.3. Heavy metal levels

Heavy metal levels in *F. aquilonia* are given in Table 3. The levels were higher in the polluted area for all metals, except for Hg, which showed no difference between the areas (Table 3). The largest relative differences between the study areas (polluted/unpolluted) were found for As (4.1), Ni (2.4), Cu (2.1) and Pb (1.8), which are metals that belong to the main pollutants of the smelter. Typically, these metals show exponential decrease with increasing distance to the smelter, as shown for As in Fig. 2. Cluster analysis on correlations among different

Table 3
The mean (±SE) heavy metal concentrations (ppm, d.w.) in the samples of *Formica aquilonia* workers at two zones around the pollution source

| Metal | *n* | Polluted area | | Unpolluted area | | *F* | *P* |
|---|---|---|---|---|---|---|---|
| | | $\bar{X}$ | SE | $\bar{X}$ | SE | | |
| Al | 36 | 51.2 | 3.80 | 49.7 | 8.70 | 4.20 | 0.048 |
| As | 37 | 1.34 | 0.14 | 0.33 | 0.03 | 135.8 | <0.0001 |
| Cd | 37 | 10.1 | 0.52 | 7.67 | 0.38 | 14.5 | 0.0006 |
| Cu | 37 | 42.9 | 2.90 | 20.5 | 0.83 | 97.1 | <0.0001 |
| Hg | 37 | 0.21 | 0.03 | 0.23 | 0.01 | 1.76 | 0.19 |
| Ni | 37 | 8.77 | 0.72 | 3.67 | 0.24 | 82.4 | <0.0001 |
| Pb | 37 | 1.82 | 0.19 | 1.02 | 0.07 | 21.5 | <0.0001 |
| Zn | 37 | 550 | 13.7 | 507 | 15.7 | 4.80 | 0.035 |

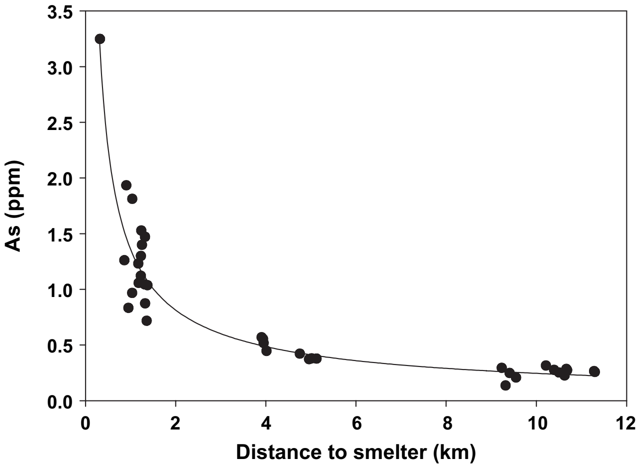

Fig. 2. Arsenic levels (ppm, d.w.) in workers of *Formica aquilonia* collected from the mound surface at different distances from the pollution source ($n = 37$).

metals shows that concentrations of these four metals are strongly correlated, while Cd, Zn and Hg seem to behave in a different way (Fig. 3). The distribution of Al concentrations in our samples was most distinctive, showing no correlations with the other metals (Fig. 3).

### 3.4. Morphometric measurements

Body mass, head width and residual mass of *F. aquilonia* workers did not significantly differ between the study sites (mixed-model nested ANOVA, body mass: $F_{1,35} = 0.04$, $P = 0.84$; head width: $F_{1,35} = 0.04$, $P = 0.85$; residual mass: $F_{1,35} = 2.93$, $P = 0.096$; $n = 366$ individuals). There were significant differences in all these traits among mounds (mixed-model nested ANOVA, body mass: $Z = 3.01$, $P = 0.0013$; head width: $Z = 2.96$, $P = 0.0016$; residual mass: $Z = 2.29$, $P = 0.011$) but this variation was unrelated to the pollution level. There were no correlations between morphological traits and timber volumes (coniferous or deciduous) or heavy metal (Al, Cu, Cd, Ni, Zn, As, Pb, Hg) levels (Pearson correlations, $P > 0.05$ in all tests). Only one individual, collected from the polluted area, of 367 studied showed the labial gland syndrome.

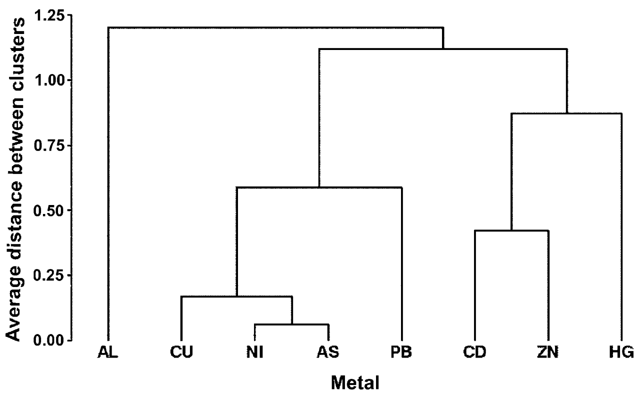

Fig. 3. Cluster analysis on correlations between the levels of different heavy metals in *F. aquilonia*.

## 4. Discussion

Along the pollution gradient of a copper smelter, two *Formica* species showed changes in their relative abundance, which could not be explained by habitat characteristics: *F. lugubris* was less abundant and *F. rufa* was proportionally more abundant in the polluted area than in the unpolluted area. The number of sampled nests mound was, however, relatively small in these two species. The two dominant species, *F. aquilonia* and *F. polyctena*, showed no difference in their relative abundance between the study areas, but showed different habitat preferences. *F. aquilonia* is known to prefer old forest (with high timber volume) while *F. polyctena* is known to prefer sunny forest edges (Adlung, 1966; Punttila, 1996). Also *F. pratensis* prefers relatively open and patchy forests, which are typical to the polluted areas. Our data showed that habitat characteristics were more important determinants of occurrence than pollutant levels in these three species.

In addition to pollutant levels, our study covered one important habitat component, i.e. the timber volumes of deciduous and coniferous trees. Timber volume has relevance for wood ants because it indicates the level of forest succession (forest age), canopy cover and, more specifically, amount of available foraging substrate (Rosengren and Sundström, 1991). Several other factors might still affect the ant community structure. They are, for example, forest fragmentation (Punttila, 1996), humidity (North, 1991) and soil characteristics (Lorber, 1982). However, our study sites were selected so that they would represent a similar forest type, i.e. relatively barren pine dominated forests typical of the study area. This means that there were no major differences among the study sites in relation to soil moisture or productivity. Continuous forest stands in the polluted area tend to be smaller than those in more distant areas, but it has been found that fragment size has a minor effect on ant community structure (Punttila et al., 1994). It should also be recalled that many changes in habitat, like the ground layer vegetation cover, are secondary effects of long-term pollution.

The decreased abundance of *F. lugubris* in the polluted area may be due to lower tolerance of this species to heavy metal pollution, due to some habitat characteristics not measured in our study or due to different dietary requirements compared to the other species. Because of long-term pollution, the forest canopy is relatively sparse and sites near the pollution source tend to be more exposed to sunlight and show higher temperature variation than those in the more distant forests (T. Eeva, unpublished data). However, this species is known to favour sunny woodland rides and clearings and it does not seem to be dependent on old forests. We also consider food limitation unlikely since aphids, the main food source for wood ants, are typically abundant in polluted areas and this is also the case in Harjavalta (Heliövaara and Väisänen, 1990). *F. lugubris* is classified by the IUCN (2003) as globally "near threatened" species. Proposed factors causing population decline include loss of suitable woodland habitats, urban or industrial development and intensive afforestation with conifers or destructive felling operations (UK Biodiversity Group, 1999). Comparisons of heavy metal levels in different wood ant species should be done to find out whether this species is especially prone to accumulate pollutants.

On the basis of our data, *F. rufa* could be more resistant to the effects of heavy metal pollution than the other four species. Sparse and sunny forests in the polluted area probably favour this species. In Finland, *F. rufa* is a monogynous species (only one queen in nest). It has been suggested that monogynous species are generally better adapted to unstable environments than polygynous species (Punttila, 1996). This does not, however, explain why another monogynous species, *F. lugubris*, decreased in the polluted area. The distribution patterns of *F. rufa* and *F. lugubris* may also be affected by historical factors, such as competitive exclusion of species after stochastic colonisation of a forest stand. Multiple sampling sites should, however, diminish this possibility in our study. Although the mound abundance was differently related to pollution levels in different species, the smaller size of colonies (see also Wuorenrinne, 1989) suggests that heavy metal pollution and/or pollution-related habitat changes pose an extra environmental stress for ants. To our knowledge, there are no studies on possible differences in metal accumulation or heavy metal tolerance among *Formica* species.

Of the eight metals measured, the levels of As, Ni, Cu and Pb were most strongly correlated to the distance to the pollution source. This is expected because they belong to the main pollutants of the smelter. The concentrations of these metals in *F. aquilonia* workers in the polluted area were relatively high compared to our background area or compared to the values given in literature for non-smelter sites (Nuorteva, 1999; Ukonmaanaho et al., 1998). However, much higher concentrations have been reported at smelter sites. For example, over 1000 times higher Pb concentrations (*F. pratensis* and *F. polyctena*), 14 times higher Cu concentrations (*F. polyctena*) and 9 times higher As concentrations (*Formica* sp.) have been found (Bengtsson and Rundgren, 1984; Kuehnelt et al., 1997; Rabitsch, 1995).

Although Cd and Zn are also emitted from the smelter, they showed a distinctive pattern compared to the main group of pollutants. Our data showed that concentrations of these two metals in ants were strongly correlated but varied more independently of the four other metals (As, Ni, Cu, Pb) emitted by the smelter. The main food (94%) of red wood ant workers is phloem sap excreted by aphids (Rosengren and Sundström, 1991). Conifer phloem is known to contain considerable amounts of some heavy metals, especially Cd and Zn (Nuorteva, 1990; Ylä-Mononen et al., 1989). Both metals are found together in natural deposits, they have a relatively high mobility in the soil–plant system and they are easily taken up by plants (Jarvis et al., 1976; Pimentel, 1994) and transferred to ants via aphid honeydew (Nuorteva, 1990; Stary and Kubiznáková, 1987). The emissions of Al and Hg from the smelter are small and no clear gradient was observed for these metals.

Negative correlations between body mass and high metal levels in wood ants have been reported in literature (Rabitsch, 1997). Our study did not reveal any association between heavy metal levels and morphological characters of *F. aquilonia* workers, probably due to lower levels of heavy metals in our study area. On the basis of our study, red wood ants are relatively tolerant to the heavy metal pollution. In polluted environments, red wood ants are known to benefit from their hierarchical social system: heavy metal levels have been observed to decline along the social food chain from foragers to brood and queens (Maavara et al., 1994; Martin et al., 1999). This is a probable reason for which *Formica* s. str. species seem to tolerate relatively high amounts of heavy metals in their environment and are still able to maintain reproducing colonies even in heavily polluted areas. However, more information is needed on the reasons for the variation in heavy metal levels and sensitivity among species.

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