

# Cadmium in insects after ash fertilization

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**Abstract** Ash fertilization of forests returns nutrients to forest ecosystems and has a positive effect on soil pH, but it also may elevate Cd concentrations of forest biota. Cadmium concentrations of some forest insects (*Formica* ants, carabids and Coleopteran larvae from decaying wood) were investigated in southern Finland, where two plots were fertilized with wood ash, while two other plots represented unfertilized control plots. In ants, mean Cd concentration was  $3.6 \pm 1.4$  mg/kg, with nest workers having significantly higher concentrations than workers trapped in pitfall traps. Concentrations at fertilized and unfertilized plots were similar. In carabid beetles, the average Cd concentration of *Carabus glabratus* was  $0.44 \pm 0.36$  mg/kg, with no significant difference between control plots and fertilized plots. In another carabid beetle, *Pterostichus niger*, mean Cd concentration was higher at fertilized plots compared to control plots. We conclude that the variation of Cd concentrations in the insects studied is more efficiently controlled by species-specific differences than fertilization history of the forest floor.

**Key words** bioaccumulation, cadmium, Carabidae, Coleoptera, fertilization, *Formica*, wood ash

## Introduction

More than 100 000 tons of wood ash are generated yearly in the Finnish wood industry. Ash fertilization of forests has been proposed as a solution to the problem of disposing of this ash, as it returns nutrients to forest ecosystems and has a positive effect on soil pH. So far ash fertilization has been carried out in Finland on a limited scale. One disadvantage with this procedure is the often high concentration of Cd in wood ash. As the Cd concentrations in wood ash typically vary between 1 and 20 mg/kg (Korpilahti *et al.*, 1998) ash application may contribute significantly to the stock of Cd in soil.

Adaptation to metals is a process whereby the individuals of a population, through natural selection, become adapted to survive and reproduce in a contaminated environment. Chronic exposure to toxic metal concentra-

tions over generations may potentially result in adaptation through decreased metal uptake rate and/or increased excretion rate.

There are considerable inter- and intraspecific differences in Cd accumulation within insects. Within a soil ecosystem, large differences in Cd concentrations have been observed in arthropod species collected at a single site (Heliövaara & Väisänen, 1993; Nummelin *et al.*, 2007). Cadmium is toxic to all organisms when it reaches the target where toxic effects could be manifested. Cadmium contamination of terrestrial ecosystems may upset normal ecological processes by altering the relative abundance and diversity of species. Metals in general can cause both physiological and genetic changes in species characteristics (Klerks & Weis, 1987). Metal adaptation may be achieved within a few generations, at least under laboratory conditions (Posthuma & van Straalen, 1993). Individuals can also change their behavior and avoid or reject food with a high metal concentration (Behmer *et al.*, 2005).

The purpose of the present investigation was to study the concentrations of Cd in some abundant forest insects in areas where wood ash fertilization has been applied and in corresponding control areas.

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## Material and methods

### Study area and treatments

The study was carried out in Evo, Southern Finland (61° 14' N, 25° 12' E) at drainage areas of two small lakes. Two plots were fertilized with wood ash originating from the pulp mill in Äänekoski. The ash was spread manually and quite unevenly in February 1988 at average 4 800 kg dry ash per hectare. The mean Cd concentration of the ash was 9.2 mg/kg which resulted in a Cd burden of 44 g/ha. Before treatment the Cd concentration in top soil (0–5 cm) was 0.3–0.5 mg/kg dry wt, which corresponds to 25–45 g/ha. After treatment, in 1999 the total Cd concentrations were 0.92 and 1.55 mg/kg on mineral and peat soils respectively. Two and a half years after treatment these concentrations had decreased to 0.38 and 0.40 mg/kg (Pihlström *et al.*, 1999; Kepanen *et al.*, 2005). The treatment resulted in a rise of pH from 4.4 to 5.8 in mineral soils and from 4.5 to 6.1 in peat soils (Pihlström *et al.*, 1999). We collected samples from two fertilized and two control plots.

### Insect samples

Samples were collected from June to early October 1999. The results may thus possibly be affected by seasonal variations in environmental Cd concentration (cf. Lodenius, 2002; Lodenius *et al.*, 2002). Insects were collected both passively with pitfall traps and manually. The pitfall traps were 0.5 L open plastic containers with smaller containers inside. These contained approximately 1 dL of ethylene glycol (HOCH<sub>2</sub>CH<sub>2</sub>OH) and water (1:1). Forty pitfalls were placed, 10 per study plot, in five rows with 5 m between rows and 5 m between pitfalls. Insects were collected from the pitfalls at 1–2-week intervals. As the insects were killed immediately after they were caught, the results may reflect Cd burdens of ingested food. Metal contamination was avoided by using plastic equipment.

From the pitfall material we chose for Cd analyses two carabid species (*Pterostichus niger* Schaller and *Carabus glabratus* Payk.) and one ant species (*Formica aquilonia* Yarr). In addition, we collected samples manually from dead pine trunks and ant nests situated at one fertilized and one control plot. These samples included four species of coleopteran larvae: *Rhagium inquisitor* (L.) (Cerambycidae), *Acanthocinus aedilis* (L.) (Cerambycidae), *Thanasimus formicarius* (L.) (Cleridae) and *Pytho depressus* (L.) (Pythidae). *Thanasimus* larvae are predators of bark beetles, whereas the other species feed on phloem and wood of decaying trees.

### Analytical procedures

Samples were frozen as soon as possible and stored frozen until dried overnight at 110 °C. Bigger specimens were weighed and analyzed individually while smaller specimens were pooled to samples of 0.05–0.2 g in size.

Two mL of nitric acid (HNO<sub>3</sub>, Aristar; BDH Laboratory Supplies Ltd, Dorset, UK) was added, whereafter the solutions were heated for 2 h at 50 °C and 16 h at 110 °C. After addition of 2 mL of H<sub>2</sub>O<sub>2</sub> the heating was continued for 6 h at 110 °C (standard procedure SFS-5075). The solutions were filtered and diluted to 10 mL or 25 mL. The metal analyses were carried out by using ETAAS (Varian SpectrAA 400 + GTA-96). All concentrations and weights reported refer to dry weight. The accuracy of our method was tested with a standard reference material (bovine liver; NIST SRM 1577a, with certified Cd concentration of 0.44 ± 0.06 mg/kg for which we obtained 0.45 ± 0.01 mg/kg). Our detection limit was 0.01 mg/kg.

For statistical evaluation we used analysis of variance (ANOVA), Kruskal-Wallis test, linear regression and correlation analyses using Statistix 8.0 software.

## Results and discussion

### Ants

Our ant material consisted of 26 pitfall samples of *Formica aquilonia* workers and 19 manually collected nest samples. The pitfall ants were workers leaving the nest or returning to it while the nest samples contained workers from the nest surface. In ants, different groups have been shown to accumulate different amounts of Cd with highest concentrations observed in workers and lowest in breeding males and queens (Maavara *et al.*, 1994). In our material the overall Cd concentration in ants was 3.6 ± 1.4 mg/kg (range 1.7–6.4 mg/kg; all concentrations in dry weight). The nest workers had significantly higher concentrations ( $P < 0.001$ ) than the pitfall workers (Table 1), probably indicating Cd accumulation in the nest. In contrast, Stary and Kubiznakova (1987) reported that *Formica* workers returning to the nest in a polluted area contained more Cd than workers leaving the nest. Also Migula and Glowacka (1996) have observed the highest Cd concentrations in *Formica polyctena* foragers returning to the nest. They also reported that body burdens of Cd and some other metals diminished progressively from the surface workers to those from the inner part of the hill. Cadmium concentrations in pitfall and nest workers were also measured by Maavara *et al.* (1994), who reported similar Cd concentrations for both groups. Although metal pollution causes

**Table 1** Cadmium concentrations (mg/kg dry weight) of insects (*Pterostichus niger*, *Carabus glabratus*, *Rhagium inquisitor*, *Acanthocinus aedilis*, *Pytho depressus*, *Formica aquilonia*) from fertilized and control plots. For *Pytho depressus* the samples consist of many specimens (12 from fertilized and 98 from control plots, respectively).

Species	Description	n	Cd mg/kg $\pm$ SD	Plot
Ants				
<i>F. aquilonia</i>	Foraging	13	2.5 $\pm$ 0.32	Fertilized
	Surface worker	9	5.0 $\pm$ 0.42	Fertilized
	Foraging	13	2.4 $\pm$ 0.33	Control
	Surface worker	10	5.1 $\pm$ 0.56	Control
Carabid beetles				
<i>C. glabratus</i>	Adult	9	0.39 $\pm$ 0.28	Fertilized
		9	0.48 $\pm$ 0.44	Control
<i>P. niger</i> , female	Adult	22	0.84 $\pm$ 0.40	Fertilized
		22	0.54 $\pm$ 0.45	Control
<i>P. niger</i> , male	Adult	26	0.77 $\pm$ 0.76	Fertilized
		18	0.49 $\pm$ 0.63	Control
Coleopteran larvae				
<i>R. inquisitor</i>	Larva	27	2.80 $\pm$ 1.60	Fertilized
		8	2.40 $\pm$ 0.19	Control
<i>A. aedilis</i>	Larva	20	0.94 $\pm$ 0.61	Fertilized
		39	1.30 $\pm$ 1.30	Control
<i>P. depressus</i>	Larva	2	3.90 $\pm$ 0.90	Fertilized
		11	5.60 $\pm$ 0.71	Control

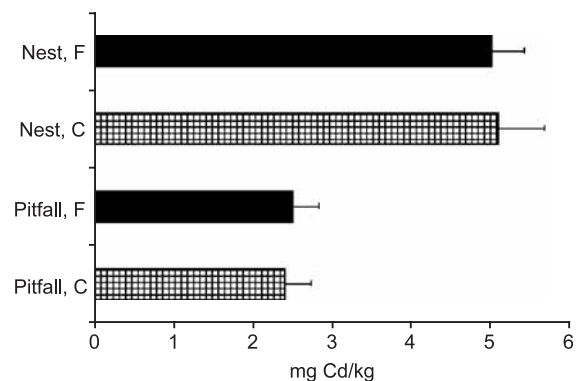
increased concentrations and reduced nest mound volumes, red wood ants can tolerate relatively high amounts of heavy metals and also maintain reproducing colonies in heavily polluted areas (Eeva *et al.*, 2004).

However, measuring Cd concentrations in ants can be problematic. Contradictory results may be related to differences between species but also to temporal fluctuations of metal concentrations. The metal concentrations in *Formica pratensis* ants, collected at sites with different contamination histories, were found to differ significantly between the measured months (Rabitsch, 1997).

Our results showed no significant differences in Cd concentrations between the fertilized and control plots (Fig. 1) indicating that the concentrations of the ants are not related to the ash fertilization.

#### Carabid beetles

In our study, the average Cd concentrations of *Carabus glabratus* specimens from fertilized and control plots were very low,  $0.44 \pm 0.36$  mg/kg ( $n = 9$ ) and  $0.05 - 1.5$  mg/kg ( $n = 9$ ), respectively. The concentrations did not differ significantly between control plots (0.48 mg/kg) and fertilized plots (0.39 mg/kg) (Fig. 2). The mean weight of *C. glabratus* specimens was  $0.28 \pm 0.07$  g and there were no significant differences in mean weights between beetles collected



**Fig. 1** Cadmium concentrations (mg/kg, means and standard deviation) of *Formica aquilonia* workers from pitfall traps and nest surfaces. Pitfall material represents workers leaving the nest or returning to it while the nest samples represent workers on the nest surface. C = control, F = fertilized.

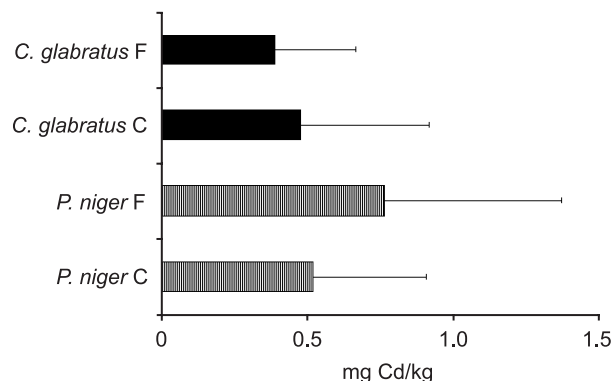
from fertilized and control plots ( $0.30 \pm 0.07$  g and  $0.26 \pm 0.02$  g, respectively).

In our material of *Pterostichus niger*, mean Cd concentration was also low for all specimens (mean  $0.66 \pm 0.54$  mg/kg;  $n = 92$ ) but the values ranged widely from 0.06 to 3.4 mg/kg. The mean concentration was higher (Kruskal-Wallis test;  $P = 0.01$ ) at fertilized plots ( $0.77 \pm 0.76$  mg/kg)

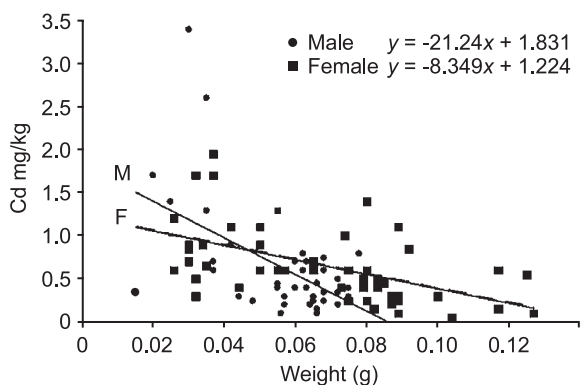
compared to control plots ( $0.49 \pm 0.32$  mg/kg; Fig. 2). There were no significant differences in Cd concentration between females and males. In a polluted area in Sweden, Lindqvist and Block (1997) found on average more than 1 mg Cd/kg from the very same species. At higher pollution levels (3.2–17 mg/kg in insects) Migula *et al.* (2004) reported a positive correlation between Cd and antioxidative enzyme activity in *Pterostichus oblongopunctatus*.

The mean weight of *P. niger* specimens was  $0.06 \pm 0.02$  g, with no significant difference between fertilized and control plots. However, there was a significant ( $P < 0.01$ ) negative correlation between body weights and Cd concentrations of the specimens, with somewhat different slopes for males and females (Fig. 3).

Predatory carabid beetles living in metal-contaminated areas may be exposed to elevated levels of metals within



**Fig. 2** Cadmium concentrations (mg/kg dry weight) of carabid beetles *Pterostichus niger* and *Carabus glabratus*; C = control, F = fertilized.



**Fig. 3** Relation between body weight and Cd concentrations (mg/kg dw) of males ( $n = 44$ ) and females ( $n = 48$ ) of *Pterostichus niger*. The negative regressions are statistically significant ( $P < 0.001$ ).

their diets. However, when compared to other second-order consumers, they have one of the lowest observed levels of metals, indicating methods of detoxification to deal with such toxicants (Mozdzer *et al.*, 2003). Heliövaara and Väisänen (1990) found Cd concentrations twice as high in the diprionid *Neodiprion sertifer* compared to its food. In an experimental food chain with contaminated food, Scheifler *et al.* (2002) demonstrated that the transfer of Cd to larvae of the carabid beetle *Chrysocarabus splendens* was very low, indicating a very effective regulation capacity. However, in their study Cd concentrations in highest exposed groups were higher (0.63 and 0.53 mg/kg in larvae and adults, respectively) than those found in control groups (0.13 and 0.01 mg/kg in larvae and adults, respectively). Cadmium concentrations in adult beetles were lower than in larvae, showing a loss of Cd during larval development, but no clear sublethal effects of the contaminated food were found.

#### Coleopteran larvae

The Coleopteran larvae of our study represented species associated with decaying wood. *Rhagium*, *Acanthocinus* and *Pytho* larvae feed in the phloem of Scots pines that have recently died, while *Thanasimum* larvae are predatory on bark beetles. The Cd concentrations in the beetle larvae were rather high, although without significant differences between plots. In *Rhagium inquisitor* and *Acanthocinus aedilis*, Cd concentrations did not differ significantly between fertilized plots and control plots (Table 1). For *A. aedilis* specimens, Cd concentrations did not correlate significantly with the weights. As far as we are aware, no studies of the metal concentrations of these beetle species have previously been published.

The mean Cd concentration of predatory *Thanasimum formicarius* was  $4.0 \pm 0.21$  mg/kg ( $n = 3$ ). In *Pytho depressus*, the mean concentration was  $5.3 \pm 0.95$  mg/kg ( $n = 13$ ) with no significant difference between fertilized and control plots.

Ants (*F. aquilonia*) and phytophagous coleopteran larvae (*R. inquisitor*, *A. aedilis* and *P. depressus*) had higher Cd concentrations than predatory carabid adults (*P. niger* and *C. glabratus*; Table 1). This may be related to metal excretion, for example, through the meconium (Gintenreiter *et al.*, 1993), or efficient detoxification in predatory adults or behavioral adaptation to avoid high concentrations.

In an experimental feeding test by Lagisz *et al.* (2005), in which contaminated food was given for 10 weeks to carabid (*Pterostichus oblongopunctatus*) larvae originating either from a chronically polluted or a control site, adaptation to metals was shown to be a slow process. During their experiment, both internal concentrations of



Cd and Zn and respiration rates of the animals were measured, but no significant differences in metal accumulation and excretion patterns or respiration rates between the populations were observed. They concluded that adaptation had not occurred in the beetles chronically exposed to toxic metal concentrations. Kramarz (1999) reported significantly increased concentrations of Cd in beetles (*Poecilus cupreus*) fed Cd-contaminated food but it seemed that these carabids were able to eliminate excess metal quickly after switching to uncontaminated food.

According to Mozdzer *et al.* (2003), larvae of *Pterostichus oblongopunctatus* show reduced growth and survival rate when fed with Cd- and Zn-contaminated food. Although Lagisz and Laskowski (2007, 2008) observed increased susceptibility to environmental stressors, decreased egg hatch and decreased body mass of young imagoes of *P. oblongopunctatus* as results of metal pollution; they found no pollution effects in the second generation. Also Stone *et al.* (2001, 2002) found decreased tolerance to food deprivation and insecticide in *P. oblongopunctatus* exposed to heavy metals, but they did not find clear trends in the occurrence of detoxification enzymes.

Adaptation itself is difficult to demonstrate, especially with carabids having very effective regulation capacity. It seems that the variation of Cd concentrations in the insects studied is more efficiently controlled by species-specific differences than fertilization history of the forest floor. Although Janssen *et al.* (1991) report that Cd assimilation is positively correlated with food assimilation in soil arthropods, other authors claim that species-specific differences in assimilation and excretion efficiencies are more important than trophic position (e.g., Laskowski & Maryanski, 1993; Posthuma & van Straalen, 1993).

Large variation in occurrence of Cd and other metals has repeatedly been observed even for closely related groups. In a study around a large metal works, van Straalen *et al.* (2001) found that Cd concentrations measured in invertebrates showed considerable variation between individual species. Concentrations were high for example in carabid beetles, but there was no relationship between the trophic position of a species and its metal-accumulating ability. Their results show the extremely complicated relationship between metal residues in invertebrates and Cd concentrations in soil. Concentrations in the soil do not necessarily explain the differences, but species-specific feeding mechanisms and metal physiologies seem to be the main determinants.

## Conclusions

The present results indicate large differences in Cd occurrence in forest insects. Although ash fertilization had

increased the Cd burden in the studied forests, the concentrations measured in insects were rather low in comparison to the polluted areas. In our study area, the time span from the application of ash fertilization (1988) until the present analyses (1999) is too short to demonstrate insect adaptation to metals.

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