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Concentrations of Heavy Metals in the Food, Faeces, Adults, and Empty Cocoons of *Neodiprion sertifer* (Hymenoptera, Diprionidae)

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Heavy metals have an adverse effect in polluted forest ecosystems situated in the vicinity of industrial plants and smelters, but little is known about their accumulation along food chains. For instance, terrestrial invertebrates may have highly variable heavy metal concentrations depending on their nutrition, trophic level, length of life cycle, systematic position etc. (Beyer et al 1985, van Straalen and van Wensem 1986, Heliövaara et al 1987) In some studies, distinct accumulation has been observed from one trophic level to another, while in others no accumulation has been recorded. Insects can excrete heavy metals directly in the faeces, or avoid food containing high concentrations. They may also excrete these elements during metamorphosis in the larval skins including the gut epithelium, pupal remnants, cocoons, gall-walls, or in the droplet excreted by the imago just after hatching.

Neodiprion sertifer (Geoffroy), the European pine sawfly, has mass-outbreaks at approximately ten-year intervals. It is a severe defoliator of Scots pine (*Pinus sylvestris* L.), usually exploiting only the previous years' needles. Eggs are laid in autumn, and the species overwinters at the egg stage in the needles. Larvae live gregariously in groups of 30-50 larvae (Pschorn-Walcher 1982). The aim of the present study was to analyse the proportion of copper, iron, nickel and cadmium in newly hatched adult insects, in their larval nutrition, faeces and empty cocoons. Larvae of *N. sertifer* were reared for this purpose on needles of varying heavy metal levels.

MATERIALS AND METHODS

The main body of data was obtained by rearing larvae of *N. sertifer* in the laboratory. The food for the larvae used in rearing was collected in the surroundings of the industrial town of Harjavalta, southwestern Finland (61°20'N, 22°10'E) in 1987. The deposition of heavy metal pollutants forms a slowly decreasing gradient.

Side branches bearing previous year's needles were collected from six pines growing on the sample plots. The plots were situated at approximately logarithmic distances along two 9-km-long transects, nine

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plots on each transect. The branches were transferred to the laboratory and placed in small plastic jars half-filled with water. The jars were transferred to larger glass pots, covered by fine nylon netting to prevent the larvae from escaping.

Egg clusters of *N. sertifer* were collected in southeastern Finland in May 1987. Larvae were reared from the 1st instar to the adult stage in the laboratory in the rearing pots. The larvae were reared in colonies of 20 larvae in each group, one colony per pot. The offspring of each female were reared on the needles collected at all the different distances from the factory complex. The pots were randomly arranged, and their position changed weekly in order to distribute the light more evenly. The branches were replaced once a week throughout the rearing period. Each colony received its nutrition from the same pine. The rearing arrangement has been described in detail in Heliövaara and Väisänen (1989).

Levels of copper, iron, nickel and cadmium were analysed from needles, faeces, adults and empty cocoons. All the samples were dried at 105°C before analysis. Approximately 1 g of needles was dry-ashed at 460°C for four hr. The ash was extracted with 10 ml concentrated HCl (p.a.). The volume was reduced to 5 ml by evaporation, and the samples then filtered and diluted to 25 ml with distilled water.

Faeces of the larval colonies were collected during their developmental period. Approximately 0.5 g of fecal pellets were digested in 6 ml of concentrated HNO_3 (Suprapur or Aristar) in a heated aluminium block. The tempreraturе was kept at 50°C for two hr, at 110°C for 16-18 hr and finally at 170°C for three hr. The digested samples were filtered and diluted to 25 ml with distilled water.

After the larvae had pupated they were transferred to a temperature of + 10°C for four wk. After being brought into the laboratory, the hatched females and their empty cocoons were collected and analysed separately. The samples were digested in concentrated HNO_3 (Suprapur or Aristar) (1 ml/100 mg of sample) in a heated aluminium block. The temperature was kept at 50°C for two hr and at 110°C for 16-18 hr. 1 ml of H_2O_2 was added and the temperature was kept at 110°C for six hr. The digested samples were filtered and diluted with distilled water to 5 or 10 ml.

The metal contents of all sample sets were measured by flame and flameless atomic absorption spectrometry (Varian SpectrAA 40 and Perkin Elmer 360).

RESULTS AND DISCUSSION

The heavy metal concentrations of *Neodiprion sertifer* adult females, their larval food and faeces, and empty cocoons containing the last larval skin gradually decreased with increasing distance from the industrial plants (Fig. 1). The power curves for each metal (Table 1) well describe the pattern in the needles and faeces, as well as in the case of cadmium in adult females.

The highest concentrations for each metal in every item analysed are given in Table 2. The heavy metal levels were higher in the needles than in the faeces or insects, except in the case of cadmium which accumulated in the

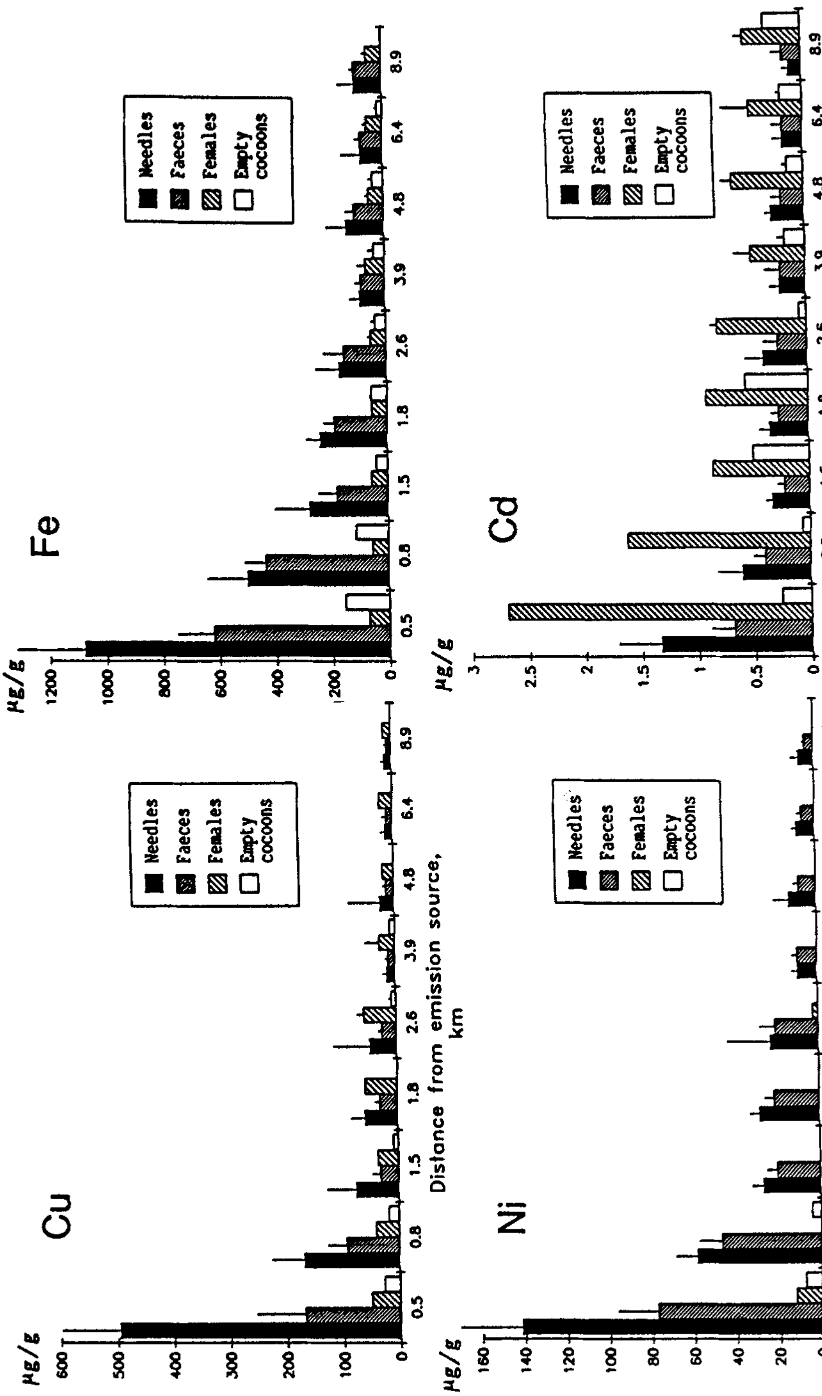

Figure 1. Concentrations (mean, halved standard deviations) of copper, iron, nickel and cadmium in the needles and adults, faeces and empty cocoons of *Neodiprion sertifer* at different distances from the emission source.

insects. The cadmium concentration in the insects was about twice as high as that in their food intake, and much higher than in their faeces. The levels in the empty cocoons were relatively low.

N. sertifer adults collected near the copper smelter contained relatively high levels of heavy metals. Unhatched cocoons from the sample plot nearest to the emission source contained 83 $\mu g g^{-1}$ Cu, 99 $\mu g g^{-1}$ Fe, 7 $\mu g g^{-1}$ Ni, and 2 $\mu g g^{-1}$ Cd (Heliövaara and Väisänen 1989). Concentrations of these heavy metals were even higher in some other pine herbivores sampled in the second year of their two-year life-cycle. *Aradus cinnamomeus* (Het., Aradidae) contained 1900 $\mu g g^{-1}$ Cu, 220 $\mu g g^{-1}$ Ni and 17 $\mu g g^{-1}$ Cd, and *Retinia resinella* (Lep., Tortricidae) 61 $\mu g g^{-1}$ Cu, 7 $\mu g g^{-1}$ Ni and 2 $\mu g g^{-1}$ Cd (Heliövaara et al 1987). Predation caused by small mammals, especially shrews, and invertebrates is a considerable mortality factor for *N. sertifer* during the pupal stage in summertime (Olofsson 1987). If heavy metals accumulate in *N. sertifer*, then further accumulation may take place along the food chain in small mammals and their predators such as birds of prey. Since *N. sertifer* is one of the most abundant forest insects, this may be an important pathway for these metals to higher trophic levels in the ecosystem.

The degree of homeostatic control which *N. sertifer* exerts over the essential trace elements, copper and iron, contrasts with that for non-essential cadmium. The cadmium levels in adult *N. sertifer* sawflies were notably elevated relative to needles. Similar results are available for some other diprionids, as well as pine moths (Heliövaara and Väisänen, *unpubl.*).

Cadmium concentrations in the food chain have also been reported varying from litter and soil to earthworms (Hartenstein et al 1980, Hunter and Johnson 1982), and other invertebrates (Hunter et al 1987). However, there are distinct differences in the accumulation of cadmium between species on the same trophic level, and the role played by the physiological equipment of the species has been emphasized (van Straalen and van Wensem 1986). Excretion of cadmium also varies between individuals of the same species (van Straalen et al 1987).

The main problem with interpreting the results is that a proportion of the heavy metals appear to have been ignored. Larval skins may contain high levels of heavy metals. but only the skin of the last instar larvae was present in the cocoons examined. It has been suggested that Collembola may get rid of significant amounts of heavy metals by shedding the gut epithelium at each moulting (Joosse and Buker 1979, van Straalen et al 1985, van Straalen et al 1987). A respective mechanism may effectively regulate the accumulation of heavy metals in *N. sertifer*, too. Another obvious pitfall is that adult females often excrete a droplet just after their emergence. This droplet might contain high levels of heavy metals. However, the results concerning unhatched cocoons do not support this hypothesis (Heliövaara and Väisänen, *unpubl.*)

The concentrations presented in this paper are mean values in all the categories. There is thus a risk that there may be differences between the needles analysed and those actually eaten by the larvae. Concentrations in the needles were only measured at the beginning of the breeding experiment, and they may have changed during the larval period. There is

Table 1. The highest concentrations of copper, iron, nickel and cadmium (µg/g) recorded in the pine needles and faeces, adult females and empty cocoons of *Neodiprion sertifer.*

	Cu	Fe	Ni	Cd
Needles	660.0	1485.5	171.2	1.8
Faeces	268.0	963.1	98.1	1.0
Females	60.5	81.8	12.5	2.7
Cocoons	28.0	157.6	7.9	0.4

Table 2. Equations for regression curves depicting the concentrations of heavy metals in the pine needles, as well as in the faeces, adults and empty cocoons of *Neodiprion sertifer* as a function of the distance from the emission source

	Equation	r	P
Needles			
Cu:	$146.50\ x^{-1.19}$	-0.971	<0.001
Fe:	$440.19\ x^{-0.85}$	-0.957	<0.001
Ni:	$52.93\ x^{-0.98}$	-0.974	<0.001
Cd:	$0.61\ x^{-0.56}$	-0.907	<0.001
Faeces			
Cu:	$68.57\ x^{-0.97}$	-0.978	<0.001
Fe:	$316.17\ x^{-0.70}$	-0.947	<0.001
Ni:	$38.21\ x^{-0.95}$	-0.989	<0.001
Cd:	$0.39\ x^{-0.40}$	-0.910	<0.001
Adults			
Cu:	$47.84\ x^{-0.77}$	-0.773	0.015
Fe:	$64.14\ x^{-0.05}$	-0.499	0.171
Ni:	-		
Cd:	$1.40\ x^{-0.57}$	-0.945	<0.001
Cocoons			
Cu:	$16.69\ x^{-0.89}$	-0.892	0.042
Fe:	$87.10\ x^{-0.67}$	-0.930	<0.001
Ni:	-		
Cd:	$0.10\ x^{-0.53}$	0.508	0.163

also a considerable amount of within-plant variation in the concentration of nutrients and secondary compounds in conifer needles (Jensen 1988). This may also be true in the case of heavy metals.

Recent studies have shown that the prevailing concentrations of heavy metals can indeed affect significantly the fecundity and egg production of *N. sertifer* (Heliövaara et al, *unpubl.*). When the larvae were reared on contaminated needles from the sites nearest to the factories, there was a 10 % decrease in weight and a 13 % decrease in the number of eggs oviposited by the females.

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