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Heavy Metals (Cd, Cu, Zn) in Wood and Wood-Feeding Insects and Other Invertebrates Associated with Decaying Pine Trees

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The study of metal distribution in trees and wood-feeding species of invertebrates is of interest from the perspective of ecological risk assessment of metal pollution as well as of forest ecology in general. Wood-feeding invertebrates are important for the physical destruction and natural removal of dead trees in forests. By carrying with them a microbial complex of wood-decomposing fungi, bacteria, protozoa, and yeasts, they contribute to the chemical process of wood decay (Ausmus 1977; Mamaev 1977). The dynamics of wood decomposition keep up energy flow and matter circulation in forest ecosystem, which are crucial in sustaining a high biodiversity in forests. At the same time, species of bark- and wood-boring beetles have been shown to accumulate heavy metals in their body tissues according to the degree of industrial pollution of the environment (Vogel 1986; Heliovaara et al. 1987; Rothz-Holzapfel & Funke 1990; Esenin 1990). As forest insects and other invertebrates function as food items for birds and mammals they are linked to the process of foodchain transfer of metals in contaminated environments.

The present study investigates the use of xylobiont invertebrates as components of a multi-species indicator system for the monitoring of forest ecosystems under metal stress. The approach followed in this study is based on the principle that each stage of decomposition of tree trunks, as shown by changes in density, hardness and chemical composition of the wood, is associated with a particular assemblage of insects and other wood-feeding invertebrates (Schimitschek 1952,1953; Mamaev 1977). The study has been carried out in two pine forest areas in The Netherlands, with different levels of metal contamination. One area, situated near the township of Budel, has been polluted by past emissions from a nearby zinc smelter; the other, a less polluted site, was situated near the city of Arnhem at about 100 km north-east of Budel. Metal contamination levels of soil and fauna of the two areas have been described earlier (Ma et al.1991). Several of the species of bark- and wood-feeding insects mentioned in this paper are studied for the first time in ecotoxicological research.

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MATERIALS AND METHODS

Sampling. The investigation was conducted in two 60 to 80 year-old pine stands in The Netherlands. Dominant tree species in both pine stands were *Pinus sylvestris* and *P. nigra,* mixed with some birch and oak. The Budel and Arnhem study areas shared a similar podzolic type of sandy soil. Sampling was carried out in October, 1994. Wood-inhabiting invertebrates were collected from under bark and the inner wood of decaying pine trees and logs over an area of about I.5 ha at each site. Species collected included Coleoptera (Cerambycidae), i.e., *Rhagium iquisitor* L., *R. bifasciatum* L., *Arholpalus (=Criocephalus) rusticus* L., *Anastrangalia revi F., and Corymbia rubra* L. In addition, sampling included Coleoptera (Elateridae), i.e., *Elater (Ampedus) sanguineus* L. and *Melanotus rufipes* Hbst.; Diptera (Stratiomyidae) i.e., *Zabrachia minutissima* Zett.; Chilopoda, i.e., *Lithobius ,forficatus;* and Lumbricidae, i.e., *Dendrobaena octaedra* Sav. Specimens used for metal analysis were selected according to a given age-weight class of each species, cerambycid larvae were also selected from head capsule dimensions. All animals were killed by freezing. Excrements were collected from the same part of the tree trunk where also wood and insect samples were taken. Samples of inner bark were cleaned from the presence of lichens, algae, and soil particles; the top layer (2-3 mm thickness) was analyzed.

Chemical analysis. Wood and bark tissues and larval frass were homogenized in an agate ball mill (Retch type S1) to a powder. All samples were dried at 50-70 °C untill constant weight and digested in HNO_3 65% (Suprapur, Merck) with gradual heating to 180 °C. Wood samples were digested in a teflon autoclave in a microwave oven. Concentrations of cadmium (Cd) were measured on a graphite-furnace atomic absorption spectrophotometer (Thermo Jarell As11 188; autosampler AS 150). Zinc (Zn) and copper (Cu) were determined with flame AAS (Instrumentation Laboratory, type Video 12). For Cd background correction was applied using Smith-Hieftje method (IL Video Manual, Allied Analytical Systems, Andover, USA, 1983) (Ma et al. 1991). Analytical quality was checked by comparison of data with those of standard reference materials from the CEC Community Bureau of References (Bovine Liver, CRM No 185) and from the International Plant-analytical Exchange (IPE), Wageningen University, The Netherlands (Douglas Fir No 994).

RESULTS AND DISCUSSION

Table 1 shows the mean concentration of Zn, Cu, and Cd measured in woody tissues of pine trunks and logs at successional stages of wood decay as characterised by typical wood-feeding species of invertebrates. In addition, data are given on metal concentrations in the frass of cerambycid larvae. According to the classification of Mamaev (1977), the first stage of wood decay is represented by stage A when the bark is attacked by, e.g., larvae of *Rhagium inquistor*.. Subsequent stages of I to V are each characterised by the presence of various xylem-feeding species of cerambycids, as is shown in Table 1. Stage VI is the final stage of decomposition when the debris of logs becomes soiled with particles

of soil through the action of specific wood-feeding species of lumbricid earthworms and other invertebrates.

Table 1. Mean concentration (mg/kg of dry weight ± SE) of metal in tissues of pine wood and frass of cerambycid larvae. Species of invertebrates representative of various successional stages of wood decay are given in brackets. Samples (N=3) were pooled from at least seven subsamples.

Sample type	Site	Zn	Cu	Cd
Stage A *(Rhagium inquisitor)*				
Phloem tissue	Budel	299 ± 16	7.1 ± 0.47	7.9 ± 0.32
	Arnhem	45 ± 5.8	9.5 ± 0.44	1.5 ± 0.10
Frass material	Budel	275 ± 15	10 ± 2.1	9.7 ± 0.53
	Arnhem	48 ± 4.3	8.6 ± 0.87	1.9 ± 0.17
Stage I *(Arhopalus rusticus)*				
Xylem tissue	Budel	37 ± 1.9	2.5 ± 0.83	2.2 ± 0.12
Frass material	Budel	44 ± 2.3	5.7 ± 1.3	4.0 ± 0.75
Stage II-III *(Anastrangalia reyi)*				
Xylem tissue	Budel	41 ± 2.1	3.7 ± 0.51	2.9 ± 0.84
	Arnhem	11 ± 1.2	4.2 ± 0.45	0.47 ± 0.05
Frass material	Budel	57 ± 0.75	7.7 ± 0.94	4.3 ± 1.2
	Arnhem	18 ± 0.49	9.7 ± 0.83	0.75 ± 0.09
Stage IV *(Corymbia rubra)*				
Xylem tissue	Budel	61 ± 3.7	4.5 ± 0.92	3.1 ± 0.76
Frass material	Budel	71 ± 4.1	11 ± 0.20	4.8 ± 1.4
Stage IV-V *(Elateridae, Carabidae, Chilopoda)*				
Xylem tissue	Budel	63 ± 9.3	4.7 ± 1.1	4.1 ± 1.1
	Arnhem	15 ± 1.2	5.3 ± 0.39	1.3 ± 0.08
Stage VI *(Lumbricidae)*				
Wood debris with soil particles	Budel	195 ± 12	11 ± 0.85	9.5 ± 1.3
	Arnhem	114 ± 9.7	12 ± 1.1	2.2 ± 0.10

Metal concentrations of Zn and Cd, but not of Cu, were higher in phloem than in xylem tissues, differences were significant for trunks of the Budel site (t-tests, $p<0.01$). Larval frass contained similar concentrations of metal as the substrate fed upon and may thus serve as an internal control of the accuray of the wood analyses. Pine trunks at the Budel site had elevated concentrations of Zn and Cd, but not of Cu, compared to those of the Arnhem area (t-tests, $p<0.05$).

Tables 2a and b show the distribution of metals in the larvae of various species of cerambycid beetles collected from pine trunks of the Budel and Arnhem areas, respectively. The larvae of *Rhagium inquisitor*, a phloem-feeding species which is found beneath the bark at the border with the sapwood, had higher tissue concentrations of Zn and Cd in comparison to the xylem-feeding species (t-tests, $p<0.05$). The difference is apparently associated with the higher concentration of these metals in phloem compared to xylem (Table 1). Concentrations of Zn and Cd were generally higher in the larval gut than in fat body tissues (t-tests, $p<0.05$). This is in agreement with the known function of the gut as a site of metal detoxication and storage in insects (Heliovaraa et al. 1987; Hopkin 1989; Krantzberg & Stokes 1990).

Table 2a. Average metal concentration (mg/kg of dry weight ±SE) in larval body tissues of cerambycid beetles collected from the Budel pine forest and associated with different stages of wood decay. The number of analyzed specimens per category was 9-15.

Insect species	Zn	Cu	Cd
Rhagium inquisitor (stage A)			
Larvae: midgut	298 ± 25	28 ± 6.8	189 ± 61
hindgut	325 ± 46	25 ± 3.7	11 ± 3.3
fat body	69 ± 21	46 ± 5.3	7.8 ± 2.1
residual tissues	201 ± 33	30 ± 9.1	10 ± 5.3
Arhopalus rusticus (stage I)			
Larvae: midgut	87 ± 27	24 ± 3.7	35 ± 8.4
hindgut	64 ± 11	42 ± 18	7.2 ± 3.1
fat body	55 ± 1.8	28 ± 8.5	1.4 ± 0.72
residual tissues	178 ± 42	31 ± 13	0.94 ± 0.38
Anastrangalia reyi (stage II-III)			
Larvae: midgut	80 ± 14	27 ± 4.1	31 ± 13
hindgut	87 ± 24	39 ± 7.1	10 ± 4.6
fat body	24 ± 1.1	29 ± 4.9	4.2 ± 1.5
residual tissues	124 ± 19	28 ± 7.6	1.8 ± 0.72
Corymbia rubra (stage IV)			
Larvae: midgut	76 ± 11	10 ± 3.5	18 ± 6.1
hindgut	328 ± 39	12 ± 3.8	4.1 ± 0.62
fat body	39 ± 9.2	23 ± 1.6	0.90 ± 0.27
residual tissues	264 ± 60	17 ± 0.36	0.42 ± 0.08

Table 2b. Same as in Table 2a, but with data for the Arnhem area. The number of analyzed specimens per category was 7-15.

Insect species	Zn	Cu	Cd
Rhagium inquisitor (stage A)			
Larvae: midgut	134 ± 38	94 ± 15	25 ± 4.6
hindgut	119 ± 30	132 ± 38	7.4 ± 1.0
fat body	25 ± 3.1	58 ± 12	1.2 ± 0.48
residual tissues	176 ± 20	33 ± 8.9	0.54 ± 0.47
Adults	129 ± 7.2	43 ± 5.2	0.61 ± 0.19
Rhagium difasciatum (stage I)			
Larvae: midgut	71 ± 12	54 ± 5.3	6.7 ± 1.8
hindgut	103 ± 28	125 ± 26	1.1 ± 0.22
fat body	29 ± 4.1	73 ± 16	0.21 ± 0.11
residual tissues	148 ± 23	22 ± 3.6	0.32 ± 0.17
Adults	126 ± 23	47 ± 4.1	0.78 ± 0.41
Anastrangalia reyi (stage II-III)			
Larvae: midgut	97 ± 27	51 ± 11	5.1 ± 0.65
hindgut	109 ± 17	111 ± 18	1.3 ± 0.20
fat body	25 ± 0.84	40 ± 9.7	0.92 + 0.25
residual tissues	143 ± 8.0	30 ± 13	0.32 ± 0.11

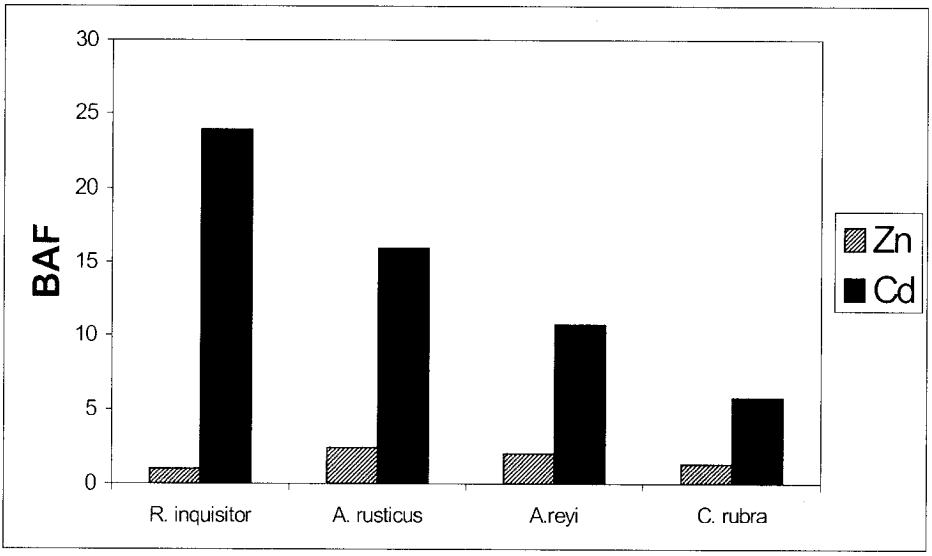

Figure 1. Bioaccumulation factor (BAF) of Zn and Cd in the larval midgut of four species of cerambycid beetles collected at the Budel site. Each species represents the following successional stages of wood decay: *Rhagium inquisitor* (stage A), *Arhopalus rusticus* (stage I), *Anastrangalia reyi* (stage II-III), and *Corymbia rubra* (stage IV).

It is further known that numbers and biomass of larval gut microorganisms are greater in insect species developing in phloem than those in xylem (Gusteleva & Isaev 1982). This fact is relevant because microbial cell walls are sites of strong metal adsorption (Plette et al. 1995). Within gut tissues, differences in metal concentration between midgut and hindgut were only significant with regard to Cd, with higher concentrations present in midgut than in hindgut (t-tests, $p<0.05$).

The variation in metal concentration of cerambycids reflected site-specific differences in metal contamination. Tables 2a and b thus show that *R. inquisitor* sampled at Budel had elevated levels of Zn and Cd compared to specimens at the Arnhem site (t-tests, $p<0.01$). This was also the case with some xylem-feeders, such as *Anastrangalia reyi*, but only so with regard to Cd. The level of Zn did not differ significantly between sites presumably because of the relatively small difference in Zn content of xylem tissues between the two sites (Table 1). Concentrations of Cu tended to be higher in cerambycids from the Arnhem site, even though the contamination of the wood was not significantly greater than at the Budel site (Table 1).

The capability of organisms to accumulate metals can be quantified by calculating the bioaccumulation factor (BAF). The BAF is defined as the ratio between the concentration of a contaminant in (part of) the organism and the concentration present in the substrate fed upon. Figure 1 shows the values of BAF as calculated for Zn and Cd in the midgut of four different species of cerambycid larvae collected at the Budel site and each representative of increasing stages of wood

decay. The value of BAF calculated for Zn remained constant within a range of about l-2 for all four cerambycid species investigated. In contrast to Zn, the BAF of Cd showed a distinct decrease from a value of 24 for larvae of *Rhagium inquisitor* (stage A of wood decay) towards a value of 6 for *Corymbia rubra* (stage IV of wood decay). The explanation for this decrease in BAF has to remain speculative at present. It may indicate the existence of a species variation in the intrinsic capacity of cerambycids to accumulate Cd. Such variation may be related to a difference in the intestinal microbial complex between species. As gut microorganisms play a key role in metal adsorption and immobilisation they would be crucial also in determining the value of BAF. An alternative hypothesis would be that the bioavailability of Cd decreases with an increasing level of wood decay. However, such possibility does not seem very likely because a similar phenomenon would be expected for other metals, which, as shown for Zn in Fig.1, is not the case.

Table 3a. Average metal concentration (mg/kg of dry weight ±SE) in wood-feeding species of invertebrates other than cerambycids collected from decaying wood. The number of specimens per category was 5-21. Data for Budel pine forest.

Invertebrate species (with stage of wood decay	Zn	Cu	Cd
Zabrachia minutissima (stage A) Larvae	326 ± 1.0	29 ± 3.4	137 ± 26
Elater sanguineus (stage III-IV) Larvae	213 ± 75	33 ± 4.1	1.3 ± 0.59
Larvae with gut removed	149 ± 9.2	21 ± 9.7	0.12 ± 0.05
Adults	148 ± 8.2	24 ± 3.2	0.10 ± 0.05
Melanotus rufipes (stage II-IV) Larvae	150 ± 18	35 ± 10	2.2 ± 0.78
Larvae with gut removed	167 ± 11	30 ± 13	0.08 ± 0.04
Litobius forficatus (stage IV-V) Whole body	608 ± 172	70 ± 19	1.8 ± 0.69
Body with gut removed	340 ± 93	43 ± 5.2	0.86 ± 0.32
Carabus violaceus (stage IV-V) Adults	83 ± 7.2	11 ± 0.74	0.25 ± 0.09
Dendrobaena octaedra (stage VI) Whole body	529 ± 112	16 ± 3.7	40 ± 17
Body with gut removed	576 ± 82	6.3 ± 0.75	20 ± 7.4

Table 3b. Same as Table 3a, but with data for Arnhem pine forest. The number of specimens per category was 5-21.

Invertebrate species (with stage of wood decay)	Zn	Cu	Cd
Elater sanguineus (stage III-IV) Larvae	154 ± 19	43 ± 14	0.21 ± 0.07
Larvae with gut removed	127 ± 10	26 ± 7.1	<detection limit
Melanotus rufipes (stage III-IV) Larvae	164 ± 19	43 ± 11	0.72 ± 0.25
Larvae with gut removed	129 ± 12	28 ± 8.2	0.19 ± 0.07
Litobius forficatus (stage IV-V) Whole body	348 ± 80	72 ± 10	1.5 ± 0.63
Body with gut removed	316 ± 52	41 ± 8.2	0.14 ± 0.04
Carabus violaceus (stage IV-V) Adults	80 ± 9.4	11 ± 0.52	0.02 ± 0.009
Dendrobaena octaedra (stage VI) Whole body	331 ± 56	17 ± 3.4	31 ± 17
Body with gut removed	445 ± 73	25 ± 13	38 ± 18

Metal concentrations present in wood-feeding invertebrates other than cerambycid insects are shown in Tables 3a and b. These invertebrates include earthworms, centipedes, dipterans, and carabids. Levels of Zn and Cd, but not .of Cu, in specimens collected from Budel were not significantly different from those of the Arnhem site; however, mean values often had relatively large standard deviations reflecting a large variability of the data. High concentrations of Cd were found in the phloen-feeding dipteran larvae of *Zabrachia minutissima* collected at the Budel site. Concentrations of Zn were relatively high in the chilopod, *Litobius forficatus,* and the wood-feeding earthworm, *Dendrobaena octaedra,* compared to lower levels found in the adult stage of the carabid beetle, *Carabus violaceus,* and the elaterid beetle, *Melanotus rufipes.*

Our data do not support the existence of some kind of relationship between metal distribution and trophic position of species, such as predators versus decomposers. This is in agreement with most other studies, as reviewed by Jansen et al. (1993). Attempts to confirm the significance of trophic level in explaining the species variation in metal distribution within assemblages of invertebrates have sofar yielded quite ambiguous results (Martin & Coughtrey 1982; Van Straalen & van Wensem 1986; Roth 1993). In retrospect, this is not very surprising because in most cases it is practically impossible to designate an exact trophic position to a given species. For instance, elaterid beetles, such as *Elater (Ampedus) sanguineus* and *Melanotus rufipes,* are facultative predators of xylobiont insects but they are necrophagous as well (Mamaev 1977). Larvae of Z. *minutissima* do not only feed on dead arthropods but also on fungal mycelia. Mycelium is known to have a large capacity to adsorb metals. Furthermore, *Carabus* frequently migrates over relatively large areas, which may result in a highly variable amount of exposure. Carabids, centipedes, earthworms, and other species of soil-dwelling invertebrates may also move frequently between litter, top soil, and the bark of decaying wood. Such behaviour would result in metal uptake from various different sources and consequently in a highly variable exposure. This kind of large heterogeneity in exposure together with the existence of a large variation in intrinsic properties of species to accumulate metals (see Figure 1) will greatly obscure the demonstration of trophic rules in metal distribution, if any.

The results of the present study show that pine trees in contaminated forest areas may accumulate heavy metals such as zinc and cadmium to a considerable extent in woody tissues. It is further concluded that insects and other invertebrates feeding on the trunks or logs may contribute substantially to heavy metal circulation in forest ecosystems in contaminated areas. Species may be selected for use in biomonitoring when accounting for the large variation in the capacity of metal accumulation among species.

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REFERENCES

Ausmus BS (1977) Regulation of wood decomposition rates by arthropod and annelid populations. Ecol Bull (Stockholm) 225: 180-192.

Esenin AV (1990) Peculiarities of the macro- and microelement accumulation by xylophages in irradiated tree-stands. In: Ecological foundations of nature protection, Part 2, Moscow, p. 111-112 (in Russian).

Gusteleva LA, Isaev AS (1982) Microflora of xylophagous insects. Novosibirsk, Nauka (in Russian).

Heliovaara K, Vaisanen R, Braunschweiler H, Lodenius M (1987). Heavy metal levels in two biennial pine insects with sap-sucking and gall-forming life-styles. Environ Pollut 248: 13-23.

Hopkin SP (1989) Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Science, London.

Jansen MPM, Ma WC, van Straalen NM (1993) Biomagnification of metals in terrestrial ecosystems. Sci Total Environ Suppl: 511-524.

Krantzberg G, Stokes PM (1990) Metal concentrations and tissue distribution in larvae of *Chironomus* with reference to X-ray micro-analysis. Arch Environ Contam Toxicol 219:84-93

Ma WC, Denneman W, Faber J (1991) Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. Arch Environ Contam Toxicol 20:266-270.

Mamaev BM (1977) Biology of insects destroying (tree) wood. Entomology, Review of Science and Engineering, Moscow, V.3, 215 p.(in Russian)

Martin MH, Coughtrey PJ (1982) Biological Monitoring of Heavy Metal Pollution. Applied Science Publishers, London.

Plette ACC, van Riemsdijk WH, Benedetti MF, van der Wal A (1995) pH dependent charging behaviour of isolated cell walls of a Gram-positive soil bacterium. J Colloid Interface Science 163:354-363.

Roth M (1993) Investigations on lead in the soil invertebrates of a forest ecosystem. Pedobiol 237:270-279.

Roth-Holzapfel M, Funke W (1990). Element content of bark-beetles *(Ips typographus* L, *Trypodendron lineatum* Oliver, Scolytidae): A contribution to biological monitoring. Biol Fertil Soils 2:192-198.

Schimitschek E (1952) Forstentomologische Studien im Urwald Rot-Wald. I. Z Angew Entomol 234: 178-215.

Schimitschek E (1953) Forstentomologische Studien im Urwald Rot-Wald.II. Z. Angew.Entomol 234:514-542.

Van Straalen NM, van Wensem J (1986) Heavy metal content of forest litter arthropods as related to body-size and trophic level. Environ Pollut 2: 209-221.

Vogel WR (1986) Zur Schwermetallbelastung der Borkenkafer. Entomol Exp Appl 2:259-269.