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Risk assessment of heavy metal pollution for detritivores in floodplain soils in the Biesbosch, the Netherlands, taking bioavailability into account

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"Capsule": *Low bioavailability reduced the impact on detritivores.*

Abstract

Floodplains of the European rivers Rhine and Meuse are heavily polluted. We investigated the risk of heavy metal pollution (Cd, Cu, Pb, Zn) for detritivores living in a floodplain area, the Biesbosch, the Netherlands, affected by these rivers. Total soil, pore water and 0.01 M $CaCl_2$ extractable concentrations and concentrations in plant leaves, earthworms, isopods and millipedes were measured in two sites and compared with literature data to assess possible risks. Based on total metal concentrations in soil, serious effects on detritivores were expected. However, 0.01 M $CaCl_2$ extractable, pore water and plant leaf concentrations were similar to metal concentrations found in unpolluted areas. Concentrations of Cu and Cd in earthworms and Cu in millipedes were higher in the Biesbosch than in animals from reference areas. All other measured concentrations of heavy metals in earthworms, isopods and millipedes were similar to the ones found in reference areas. Despite high total soil concentrations, effects of Zn, Cu, Pb and Cd pollution on isopods are therefore not expected, while millipedes may only be affected by Cu. Since Cu and Cd levels in earthworms were increased compared to animals in unpolluted soils, this faunal group seems to be most at risk. Given the engineering role of earthworms in ecosystems, effects on the ecological functioning of floodplain soils therefore cannot be excluded.

Keywords: Risk assessment; Heavy metals; Flood plains; Detritivores; Bioavailability

1. Introduction

Risk assessment of heavy metal pollution for terrestrial environments is often based on total soil concentrations and does not take the actual exposure into account (Crommentuijn et al., 2000). Therefore, risk assessment would be improved by taking availability and internal metal concentrations in receptor organisms into consideration (Chapman and Wang, 2000; Van Straalen, 1996). Basing risk assessment only on available metal concentrations in the environment of detritivores can be misleading, because, in the field, exposure routes and their relative importance are difficult to determine. Basing risk assessment only on internal metal concentrations can be misleading, too, due to regulation and/or adaptation mechanisms influencing internal metal concentrations.

The aims of this paper are to assess the risk of heavy metal pollution for detritivores in two floodplain soils in the Biesbosch, the Netherlands by analysing metal concentrations in both the environment (soil, pore water, plant leaves) and detritivores and to gain insight into exposure routes of heavy metals for these organisms. Because no reference areas could be found in the Biesbosch, it was necessary to compare our results with literature data on reference areas and effect concentrations.

Three groups of detritivores were studied: earthworms, isopods and millipedes. Detritivores were

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chosen for two reasons. Firstly, because they play an important role in the decomposition of litter and therefore are important for ecosystem functioning (Brussaard, 1998). Secondly, because little research has been conducted on detritivores in floodplains.

2. Materials and methods

2.1. Field sites, transects and sampling points

Two field sites were selected in the National Park "De Brabantsche Biesbosch" in the Netherlands: Lage Hof (LH) and Petrus Plaat Oost (PO). The dominant vegetation at both sites consists of the stinging nettle, *Urtica dioica,* and common reed, *Phragmites australis*. Both sites are shaped rectangular. PO is approximately 15 m wide and 125 m long. LH is approximately 40 m wide and 100 m long.

All samples were taken along transects situated in areas with a homogeneous vegetation. Unless mentioned otherwise, one sample was taken per sampling point. Most samples were taken to a depth of approximately 15 cm because most detritivores were expected to live in this upper soil layer. Only earthworms were sampled to a depth of 25 cm. In PO, one transect was used with a length of 100 m, situated parallel to the length of the rectangle. Five sampling points were located at a mutual distance of 25 m. In LH in spring 2001, three transects and five sampling points per transect were used for a first inventory of the field sites to determine the level of pollution. During summer 2001 and 2002, two other transects were sampled in LH, because the markings of the original transects could not be recovered. The two new transects were 80 m long and situated both parallel to the length of the rectangle and to each other at a distance of 20 m. Five sampling points per transect were used at a mutual distance of 20 m. The first inventory of LH, during spring 2001, showed that variations in soil texture and in total soil concentrations of heavy metals in this site were small (Table 1). Therefore these variables were assumed to be homogeneous and not determined along the new transects.

Table 1
Characteristics of the top 15 cm soil layers of the study areas Petrusplaat Oost (PO) and Lage Hof (LH) in the Biesbosch, the Netherlands

	PO		LH	
	n	mean±S.D.	*n*	mean±S.D.
% organic matter	5	11.4±0.6	3[a]	18.8±2.0
% inorganic carbon	5	3.2±0.2	5	2.9±0.2
% clay	5	20.5±2.6	3[a]	35.1±1.1
pH-$CaCl_2$	5	6.89±0.05	10	6.94±0.05
pH-H_2O	5	7.11±0.09	10	7.11±0.03

[a] Each consisting of 5 pooled samples.

2.2. Total concentrations of heavy metals and soil texture

In spring 2001, Edelman auger samples, Ø 7 cm, of the upper 15 cm soil layer (after removal of litter), were taken in PO and LH for the determination of total concentrations of Zn, Cu, Pb and Cd and soil characteristics. Five samples were taken per transect. The samples taken in LH were pooled per transect to reduce the number of analyses. The Edelman auger samples were analyzed by the Bedrijfskundig Laboratorium Grond- en Gewasonderzoek (BLGG) in Oosterbeek, the Netherlands. Total concentrations of heavy metals were determined according to the Dutch standard NEN 6426, clay content according to NEN 5753 and organic matter content according to NEN 5754.

2.3. Available concentrations of heavy metals and soil pH

In summer 2001, split corer samples, Ø 10 cm, of the upper 15 cm soil layer (after removal of litter), were taken in PO and LH to determine the pH-H_2O, pH-$CaCl_2$ and concentrations of heavy metals in 0.01M $CaCl_2$ extracts and in pore water. To measure the pH-H_2O and pH-$CaCl_2$, soil was dried at 40 °C for 24 h. Soil samples were shaken with deionized water or 0.01 M $CaCl_2$ for 2 h at 200 rpm, using a solution:soil (w/w) ratio of 5:1. After sedimentation of soil particles, pH was measured using a Consort p907 meter. After filtering over a Schleicher & Schuell 0.45 μm membrane, solutions were analyzed for $CaCl_2$-extractable concentrations of heavy metals. Pore water was collected by centrifuging 20 g soil, with an average moisture content of 63% in PO and 88% in LH, using a Kontron TGA-6 centrifuge. Soil was centrifuged for 45 min with a relative centrifugal force of approximately 2000 g over a Schleicher & Schuell 0.45 μm membrane filter, placed inside the tubes.

2.4. Soil invertebrate sampling

In summer 2001, split-corer samples, diameter 10 cm, were taken in PO and LH to a depth of 15 cm, including the litter layer, to determine the species richness and density of small isopods and millipedes. Quadrat samples, 25×25×25 cm, were used to determine the species richness and density of earthworms and larger isopods and millipedes. At three sampling points in PO and two sampling points in LH, two additional split-corer samples were taken to study variation in density and species richness in the proximity of a sampling point. Because this variation was as large as the variation between sampling points, it was decided to interpret the additional samples as independent sampling points, increasing the number of samples taken in PO from 5 to 11 and from 10 to 14 in LH. Soil animals were extracted from split-corer samples using a Tullgren apparatus (Van Straalen and Rijninks, 1982) during a period of three weeks per sample. Animals were collected in fixative (±75% ethanol, ±25% ether, ±0.003% formaline), identified and counted. Animals in quadrat samples were collected by handsorting, identified alive or after storage in fixative, and counted. Isopods were identified using Berg and Wijnhoven (1997), millipedes using Blower (1985) and earthworms using Sims and Gerard (1999).

2.5. Metal analysis

In summer 2001 and 2002, living animals, sampled in PO and LH, were collected on moist plaster of Paris by Tullgren extraction of split-corer samples and by hand-sorting quadrat samples. After identification animals were frozen, freeze-dried for two days and weighed to the nearest µg on a Mettler Toledo UMT2 or Sartorius 1712 MP8 balance. Prior to freeze-drying, earthworms were kept on moist filter paper for two days, to excrete their gut contents and were then frozen at −20 °C. Not all of the gut contents of arthropods will have been excreted at the time they were collected from the Tullgren. Arthropods were digested in pyrex tubes using a 7:1 mixture of HNO_3 65% (Baker Ultrex II Ultra pure) and $HClO_4$ 70% (Baker Ultrex Ultra pure). Prior to analysis, the digests were dissolved in 500 µl or 1000 µl of a 0.1 M HNO_3 solution. Earthworms were digested in a 7:3 mixture of HNO_3 65% (Riedel-de Haën) and demineralized water using a microwave (CEM MARS 5). Concentrations of heavy metals in soil extracts and animal digests were measured using a Perkin Elmer atomic absorption spectrometer (AAS), type 1100 B. Flame AAS was used if concentrations were high enough, otherwise graphite furnace AAS was used. Dolt II reference tissue was used to determine the accuracy of the analytical procedure. Average Cu concentrations were within the 95% confidence interval of certified values. Average Zn, Pb and Cd concentrations varied between minus 16.7% to plus 3.5% of certified values.

3. Results

3.1. Soil texture and concentrations of heavy metals in soil

Characteristics of the soils in the two study areas are shown in Table 1. Total soil, 0.01 M $CaCl_2$ extractable and porewater concentrations of Zn, Cu, Pb and Cd in the 0–15 cm soil layers of PO and LH are shown in Table 2. In both Tables, standard deviations were less than 18% of the average concentrations, although the use of pooled samples in LH caused smaller standard deviations.

3.2. Population density and species richness of detritivores

Table 3 shows the population density of isopods, millipedes and earthworms, expressed in terms of individuals and biomass per square meter.

Table 2
Total, estimated background, 0.01M $CaCl_2$-extractable and pore water concentrations (mean ± sd) of Zn, Cu, Pb and Cd in the 0–15 cm soil layer of the study areas Petrusplaat Oost (PO) and Lage Hof (LH) in the Biesbosch, the Netherlands. Also included are metal concentrations in leaves of the dominant plant species *Urtica dioica*

	Number of samples		Zn		Cu		Pb		Cd	
	PO	LH	PO	LH	PO	LH	PO	LH	PO	LH
Total soil conc. (mg/kg dry soil)	5	3[a]	1140 ± 114	2333 ± 404	142 ± 18	387 ± 31	278 ± 24	600 ± 96	11.7 ± 1.7	19.3 ± 0.6
Estimated background conc. (mg/kg dry soil)[b]	–	–	26 ± 18	56 ± 18	18 ± 4	27 ± 4	53 ± 22	71 ± 22	0.6 ± 0.2	0.8 ± 0.1
Total soil conc. divided by background conc.	–	–	43	42	8	14	5	8	20	24
0.01M $CaCl_2$-extractable conc. (mg/kg dry soil)	10	10	0.81 ± 0.58	0.91 ± 0.54	0.25 ± 0.04	0.25 ± 0.08	< DL	< DL	0.02 ± 0.00	0.02 ± 0.01
Porewater conc. (µg/l)	5	8	97 ± 22	99 ± 11	122 ± 33	103 ± 26	< DL	< DL	1.48 ± 0.36	1.49 ± 0.17
Conc. in leaves *Urtica dioica* (mg/kg dry weight)[c]	7	24	18.0 ± 3.9	29.4 ± 9.2	9.6 ± 1.0	8.6 ± 3.0	1.5 ± 0.1	0.95 ± 0.38	0.01 ± 0.01	0.01 ± 0.02

< DL: below detection limit.
[a] Each consisting of 5 pooled samples.
[b] Calculated from clay and organic matter content according to Van Gestel et al. (1992).
[c] Data provided by Notten et al.

Table 3
Density and biomass (mean ± S.D.) of isopod, millipede and earthworm species in the study areas Petrusplaat Oost (PO) and Lage Hof (LH) in the Biesbosch, the Netherlands. The number of split corer samples in PO is 11 and in LH 14. The number of quadrat samples in PO is 5 and in LH 10

group/species/total	no./m²		mg dry weight/m²	
	PO	LH	PO	LH
Isopods				
Haplophthalmus mengii	–	9 ± 34	–	2.7 ± 10.00
Ligidium hypnorum	–	18 ± 68	–	2.8 ± 10.00
Philoscia muscorum	23 ± 52	9 ± 34	57 ± 162	29 ± 107
Trachelipus rathkii	Obs.	55 ± 139	Obs.	350 ± 889
Trichoniscoides albidus	139 ± 175	182 ± 414	33 ± 53	55 ± 124
Trichoniscus pusillus	1227 ± 2002	1282 ± 1235	417 ± 681	371 ± 420
Total density of isopods	1389	1555	507	811
Millipedes-splitcorer samples				
Brachyiulus pusillus	–	55 ± 96	–	407 ± 717
Cylindroiulus latestriatus/brittanicus juv.	–	663 ± 583	–	210 ± 184
Brachydesmus superus juv.	23 ± 52	555 ± 557	2.8 ± 6.2	78 ± 78
Iulus scandinavius	–	9 ± 34	–	129 ± 481
Polydesmus denticulatus	Obs.	Obs.	Obs.	Obs.
Total density of Millipedes-splitcorer samples	23	1282	2.8	824
Millipedes-quadrat samples				
Brachyiulus pusillus	–	3.2 ± 1.0	–	24.5
Cylindroiulus latestriatus/brittanicus juv.	–	–	–	–
Brachydesmus superus	3.2 ± 1.4	13 ± 3	11 ± 5	45 ± 11
Iulus scandinavius	–	61 ± 14	–	860 ± 89
Polydesmus denticulatus	Obs.	Obs.	Obs.	Obs.
Total density of millipedes-quadrat samples	3.2	77	11	930
Earthworms				
Lumbricus rubellus juv.	93 ± 29	43 ± 51	1402 ± 438	1062 ± 1254
Lumbricus rubellus adult	19 ± 21	10 ± 11	2135 ± 2335	516 ± 591
Eiseniella tetraedra	Obs.	1.6	Obs.	14
Dendrobaena rubida	Obs.	18 ± 19	Obs.	404 ± 436
Aporrectodea caliginosa	Obs.	Obs.	Obs.	Obs.
Total earthworm density	112	73	3537	1996

Obs.: Species observed but not counted. See text for explanation. –: Species not found.

3.2.1. Densities and biomass

Isopod densities were based on counts of individuals in split-corer samples and earthworm densities on counts in quadrat samples. Millipede densities shown in Table 3 were calculated on the basis of split-corer samples and quadrat samples separately. Split-corer samples (Tullgren-extracted) contained mainly juveniles and quadrat samples (hand-sorted) mainly adult millipedes.

In PO, 82% of the isopod biomass consisted of *Trichoniscus pusillus*. The observation of *Trachelipus rathkii* in PO in summer 2002, having a dry weight approximately 20 times higher than *Trichoniscus pusillus*, indicates that this species could comprise a substantial part of the isopod biomasss in PO, although it was present in low numbers. In LH, 46% of the isopod biomass consisted of *Trichoniscus pusillus* and 43% of *Trachelipus rathkii*.

In summer 2001 in PO, the total millipede biomass consisted of *Brachydesmus superus*. The observation of *Polydesmus denticulatus* in summer 2002, about the same adult size as *Brachydesmus superus*, indicates that millipede biomass in PO was higher than shown in Table 3. Total millipede biomass in LH may be estimated by summation of millipede densities in split-corer samples and quadrat samples, if it is assumed that individuals in quadrat samples are adults and individuals in split corer samples are juveniles. The density of adult individuals will be overestimated by this procedure, because a small number of adults was also found in split corer samples. Using this method, it is estimated that

Brachyiulus pusillus accounted for 25% of the total millipede biomass in LH and *Iulus scandinavius* for approximately 55%.

In 2001, *Lumbricus rubellus* constituted 100% of the earthworm biomass in PO and 80% in LH. The biomass of other earthworm species, first observed in 2002, was low relative to the total biomass of earthworms estimated in 2001, because they were found in relatively low numbers and their dry weight was small relative to the dominant species in PO and LH, *Lumbricus rubellus*.

3.2.2. *Comparison of biomass per species group*

Total biomass of isopods, millipedes and earthworms was 4058 mg dry weight per square meter in PO and 4560 mg dry weight per square meter in LH. Earthworms accounted for 87% of this biomass in PO and 44% in LH. Isopods accounted for 12% in PO and 18% in LH and millipedes for less than 1% in PO and for 38% in LH.

3.3. *Concentrations of heavy metals in detritivores*

Table 4 shows internal concentrations of Zn, Cu, Pb and Cd in isopods, millipedes and earthworms sampled during one or more sampling periods. From the isopod species present in both areas, only *Trichoniscus pusillus* was found in numbers high enough to reliably estimate internal metal concentrations and standard deviations.

4. Discussion

4.1. *Total soil concentrations in field sites relative to unpolluted areas*

Total metal concentrations (mg/kg) in 19 relatively unpolluted areas in the Netherlands were measured by Van Gestel et al. (1992). Using linear regression models, between 50.5–85.4% of variation in total metal concentrations in the concerning areas could be explained by variation in clay content, organic carbon content and pH-H_2O. Based on these regression equations, expected background concentrations in soils of PO and LH were calculated and compared to the measured concentrations (Table 2). It appears that the relative increase of heavy metals in PO and LH, caused by pollution of the rivers Rhine and Meuse, is highest for Zn, followed by Cd, Cu and Pb.

4.2. *Total soil concentrations in field sites relative to effect concentrations*

Results of a literature search for data on toxic effects of heavy metals on earthworms are shown in Table 5. Comparing total soil concentrations in PO and LH with these data, without taking differences in soil characteristics

Table 4
Internal metal concentrations (mean±S.D. in μg/g dy weight) in earthworm, isopod and millipede species found in the study areas Petrusplaat oost (PO) and/or Lage Hof (LH) during one or more sampling periods

	Zn				Cu				Pb				Cd			
	n	PO	*n*	LH	*n*	PO	*n*	LH	*n*	PO	*n*	LH	*n*	PO	*n*	LH
Isopods																
Trichoniscus pusillus	39	199±86	47	170±90	38	87±41	38	95±44	35[3]	12±8	17	13±10	37	21±13	17	18±11
Millipedes																
Iulus scandinavius	–	–	8	138±36	–	–	9	275±77	–	–	9	2.6±2.7	–	–	8 [1]	0.9±0.4
Brachydesmus superus juv.	4	267±183	22	423±258	3	306±271275		±								

into account, shows that litter breakdown, cocoon production, growth and survival of *Lumbricus rubellus* in both PO and LH could be affected. *Dendrobaena rubida* could have a decreased cocoon production and survival in both PO and LH., while cocoon production and growth in *Aporrectodea caliginosa* might be decreased in both PO and LH and survival decreased in LH.

4.3. Pore water concentrations in field sites relative to unpolluted areas

Pore water concentrations in 19 relatively unpolluted soils in the Netherlands, measured by Van Gestel et al. (1992). Concentrations amounted 71–1376 µg/l for Zn, 54–242 µg/l for Cu, 1–147 µg/l for Pb and 0.3–4.7 µg/l for Cd. It can be concluded that pore water concentrations in PO and LH lie within the range of values found in unpolluted areas. Therefore, no effects of heavy metals on detritivores can be expected by exposure to pore water.

It should be noted that a solution containing nutrients was added to the unpolluted soils by Van Gestel et al. (1992) before measuring pore water concentrations. This may have influenced the speciation of heavy metals. Possibly, the low pore water concentrations of heavy metals in PO and LH soils can be explained by the combination of a high pH and high clay, organic and inorganic carbon contents (Sauvé et al., 2000; Peijnenburg et al., 2001).

4.4. Plant leaf concentrations in field sites relative to unpolluted areas

Ma et al. (1992) reported heavy metal concentrations in leaves of *Urtica dioica* in a relatively unpolluted area of 42–52 mg/kg for Zn, 14–20 mg/kg for Cu, 4.0–5.7 mg/kg for Pb and 0.1 mg/kg for Cd. Metal concentrations in leaves of *Urtica dioica* in PO and LH (Table 2) were always lower than these reference concentrations, so no effects of heavy metals on detritivores can be expected from dietary exposure.

4.5. Risk assessment based on metal concentrations in the environment of detritivores

Isopods and millipedes are probably mainly exposed to metals by ingestion of polluted food and do not seem at risk in the Biesbosch. Plant leaf concentrations in the

Table 5
Total soil concentrations of heavy metals (mg/kg) and resulting sublethal and lethal effects on earthworms as found in toxicity tests in the literature

Species/endpoint	Effect	Zn	Cu	Pb	Cd	Source
Earthworms						
Lumbricus rubellus						
Litter breakdown	NOEC/no sign effect	–	63	–	–	6
Litter breakdown	sign effect	–	131–373	–	–	6
Cocoon production	NOEC/no sign effect	–	13–30	200	10	4, 6
Cocoon production	EC10	88	80	–	–	7, 11
Cocoon production	EC50	348–876	122	–	–	7, 9–11
Growth	NOEC/no sign effect	620	373	1000	–	3, 6, 9
Growth	sign effect	–	160–372	–	–	6
Growth	EC50	1301–1520	250	–	50	5, 10
Survival	NOEC/no sign effect	–	160	3000	–	4, 6
Survival	EC10	1232	–	–	–	11
Survival	EC50	728–1709	1000	–	575	3, 9–11
Dendrobaena rubida						
Cocoon production	NOEC	–	<100	<500	<100	1
Growth	NOEC	–	–	1000	–	1
Survival	NOEC	<100	–	<100	–	1
Aporrectodea caliginosa						
Cocoon production	NOEC/no sign effect	–	50	–	–	8
Cocoon production	sign effect	–	110	–	–	12
Cocoon production	EC10	206–568	27–70	–	1.86	2, 7, 11
Cocoon production	EC50	442–826	68	–	–	2, 7, 11
Growth	NOEC	–	100	–	–	8
Growth	EC50	461	82	–	68.4	2, 11
Survival	NOEC	–	500	–	–	8
Survival	EC10	1402–1417	–	–	–	11
Survival	EC50	1619–3610	640	–	540	2, 11

1 = Bengtsson et al., 1986; 2 = Khalil et al., 1996; 3 = Ma, 1982a; 4 = Ma, 1982b; 5 = Ma, 1983; 6 = Ma, 1984; 7 = Ma, 1988; 8 = Martin, 1986; 9 = Spurgeon and Hopkin, 1996a; 10 = Spurgeon and Hopkin, 1999; 11 = Spurgeon et al., 2000; 12 = Van Rhee, 1975.

Biesbosch area are low and comparable to metal levels in reference areas. Heavy metal pollution in the Biesbosch therefore seems not to pose a risk for isopods and millipedes.

Earthworms may be exposed to heavy metals in pore water, food (including plant leaves) and ingested soil particles. Saxe et al. (2001) and Vijver et al. (2003) recently demonstrated that dermal uptake of metals predominates over oral uptake, suggesting that pore water is the major route of exposure. However, both studies do not exclude some additional uptake of metals from ingestion of soil particles. Metal levels in pore water and plant leaves are comparable to levels in reference soils. Total metal concentrations in soil were, however, very high. It may be concluded that the risk for earthworms in the Biesbosch will be dependent on their ability to assimilate heavy metals attached to soil particles.

Table 6
Literature data on heavy metal concentrations (μg/g dry weight) in earthworms, isopods and millipedes that were used as control in laboratory experiments or sampled in reference sites and ranges of total soil or litter concentrations (mg/kg) in these reference sites

Species/metal	Int. conc.	Total soil conc.	Source
Isopod sp.[a]			
Zn	54–485	4–380	10–16, 18–21, 26, 27, 30, 39, 44–46, 50
Cu	92–522	0.6–52	10, 15–17, 19–21, 26, 44, 46, 50, 52
Pb	0.01–66	< 0.02–231	1, 10, 14–17, 19–21, 26, 28–30, 39, 44–46, 50, 52
Cd	1.7–31	0.1–13	10, 14–17, 19–21, 26, 30, 31, 38, 39, 45, 46, 50, 52, 54
Millipedes			
Iulus scandinavius			
Zn	152–212	—	3, 14, 49
Cu	146		49
Pb	2.6–5.5	—	3, 14, 49
Cd	0.9– < 1	—	3, 9, 49
Other species			
Zn	152–827	—	3, 7, 14, 48, 49
Cu	33–221	—	7, 22, 47–49
Pb	0.51–62	—	3, 14, 48, 49
Cd	0.2–15.4	—	3, 7, 9, 22, 48, 49
Earthworms			
Lumbricus rubellus			
Zn	260–925	5.8–416	4–8, 25, 32, 35–37, 40–44, 49, 51, 53
Cu	4.6–30	0.39–44	4–8, 25, 32, 33, 35, 37, 41, 42, 44, 49, 51, 53, 55
Pb	0.3–88	3.1–170	4–6, 8, 25, 32, 35–37, 40–44, 49, 51, 53, 55
Cd	2.7–26	0.02–2.2	4–8, 32, 35–37, 41–43, 49, 51, 53
Aporrectodea caliginosa			
Zn	267–2000	50–193	34, 43–44, 57
Cu	17–35	10–52	34, 43–44
Pb	8–93	32–166	34, 43–44, 56, 57
Cd	13–31	0.27–1	34, 43, 56, 57
Dendrobaena rubida			
Zn	100–251	172	23–25
Cu	12.9–183	< 0.5–20	2, 25, 55
Pb	5–100	3–152	2, 23–25, 55
Cd	8.7–33	< 0.01–0.14	2, 55

1 = Beeby, 1980; 2 = Bengtsson et al., 1986; 3 = Berkus et al., 1994; 4 = Beyer and Crommartie, 1987; 5 = Beyer et al, 1987; 6 = Carter et al., 1980; 7 = Carter, 1983; 8 = Corp and Morgan, 1991; 9 = Crommentuijn et al., 1994; 10 = Donker et al, 1993; 11 = Donker et al., 1996; 12 = Donker et al., 1998; 13 = Drobne and Hopkin, 1995; 14 = Graff et al., 1997; 15 = Hopkin and Martin, 1982; 16 = Hopkin and Martin, 1984; 17 = Hopkin et al., 1986; 18 = Hopkin et al., 1989; 19 = Hopkin, 1990; 20 = Hopkin et al., 1993; 21 = Hopkin and Hames, 1994; 22 = Hunter et al., 1987; 23 = Ireland, 1975; 24 = Ireland and Wooton, 1976; 25 = Ireland and Richards, 1977; 26 = Jones and Hopkin, 1996; 27 = Joosse and Van Vliet, 1984; 28 = Khalil et al., 1995; 29 = Knigge and Köhler, 2000; 30 = Köhler et al., 1996; 31 = Kratz et al., 1987; 32 = Kruse and Barret, 1985; 33 = Langdon et al., 2001; 34 = Ma, 1982a; 35 = Ma, 1987; 36 = Ma, 1989; 37 = Ma, 1992; 38 = Martin and Coughtrey, 1975; 39 = Martin et al., 1976; 40 = Morgan and Morgan, 1988; 41 = Morgan and Morgan, 1990; 42 = Morgan and Morgan, 1991; 43 = Morgan and Morgan, 1999; 44 = Paoletti et al., 1988; 45 = Prosi et al., 1983; 46 = Prosi and Dallinger, 1988; 47 = Rabitsch, 1995; 48 = Read and Martin, 1987; 49 = Roth-Holzapfel, 1991; 50 = Sørensen et al., 1997; 51 = Spurgeon and Hopkin, 1999; 52 = Van Capelleveen, 1985; 53 = Van Gestel et al., 1992; 54 = Van Straalen, 1996; 55 = Weigmann, 1991; 56 = Weisenfeld, 1989; 57 = Wright and Stringer, 1980.

[a] For isopods litter concentrations are shown instead of total soil concentrations.

4.6. Internal concentrations relative to unpolluted areas

To see if heavy metal concentrations in isopods, millipedes and earthworms in PO and LH were increased by heavy metal pollution, a literature search was conducted for internal concentrations of animals from reference areas and of animals used as control in laboratory experiments.

In the literature, no data could be found on heavy metal concentrations in the isopod *Trichoniscus pusillus*. Reference concentrations in other isopod species reported in the literature are shown in Table 6. It can be concluded that Zn, Cu, Pb and Cd concentrations in *Trichoniscus pusillus* in both PO and LH (Table 4) are within the range of reference concentrations.

Data on reference concentrations in four millipede species were reported in the literature. Zn, Pb and Cd concentrations in *Iulus scandinavius* (Table 4), only present in LH, were similar to reference concentrations reported for this species (Table 6). Cu concentrations in *Iulus scandinavius* were 1.9 times the only reference value found in the literature.

No data on other millipede species present in PO and LH were found, but comparing internal concentrations in PO and LH with reference concentrations in several other millipede species (Table 6), including *Iulus scandinavius,* shows that Zn, Pb and Cd in both PO and LH are always within ranges of reference concentrations. However, Cu concentrations in millipede species, other than *Iulus scandinavius,* are 1.4–2.6 times the highest reference value in PO and 2.5–4.4 times in LH. It can be concluded that Cu concentrations in millipedes in both PO and LH seem elevated.

Internal concentrations of earthworms in unpolluted soils are shown in Table 6. Internal Zn and Pb concentrations of *Lumbricus rubellus* in PO and LH (Table 4) were within reference intervals, but Cu and Cd concentrations in this species were, respectively, 1.1–1.6 and 1.7–2.0 times the highest reference value. In PO, Zn concentrations in *Aporrectodea caliginosa* (Table 4) were within the reference interval, but Cu concentrations were 1.6 times and Cd concentrations 2.7 times higher than the highest reference value (Table 6). *Aporrectodea caliginosa* was not found in LH. Zn concentrations in *Dendrobaena rubida* in PO and LH were 1.6–1.8 times and Cd concentrations 3.2–5.2 times the highest reference value. Cu en Pb concentrations were similar to reference intervals in both PO and LH.

The use of internal metal levels in risk assessment is only meaningful if a change in exposure to heavy metals results in a change in internal metal levels. Zn, Cu, Pb and Cd in field populations of the isopod *Porcellio scaber* (Hopkin et al., 1986, 1989), millipede species (Hunter et al., 1987; Read and Martin, 1987) and the earthworm *Lumbricus rubellus* (Corp and Morgan, 1991; Ma et al., 1983, 1997; Morgan and Morgan, 1988; Spurgeon et al., 2000) show positive relationships with litter and/or total soil concentrations. Therefore, if internal concentrations in field populations of isopod, millipede and earthworm species are similar to internal concentrations in comparable reference areas, exposure to heavy metals of these populations is expected to be low or similar to reference areas.

However, interpreting the comparisons of internal metal levels measured in PO and LH (Table 4) and values reported in Table 6 should be done carefully for a number of reasons. Ranges of reference concentrations in Table 6 are based on animals of different ages, weight and collected in different seasons. Total soil concentrations of heavy metals, litter concentrations and soil characteristics also varied between reference areas. Table 6 also contains data on species not present in PO and LH and internal concentrations of animals used as control in laboratory experiments. Next to these differences, comparison between sites is difficult because standard deviations of internal metal levels in animals, sampled at the same site, are high. This could be the result of local variation in soil characteristics (pH, %OM and% clay) that influence bioavailability of heavy metals.

Table 7
Literature data on heavy metal concentrations (μg/g dry weight) in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* that show significant changes in (sub)lethal endpoints

Species/endpoint	Effect	Zn	Cu	Pb	Cd	Source
Lumbricus rubellus						
Survival	sign.	1002	55.1	—	—	1, 3
Cocoon production	sign.	1029	—	—	—	3
Growth	sign.	—	40	—	100	2
Aporrectodea caliginosa						
Survival	sign.	572	—	—	—	4
Cocoon production	sign.	414	—	—	—	4
Growth	sign.	785	—	—	—	4

1 = Langdon et al., 2001; 2 = Ma, 1983; 3 = Spurgeon and Hopkin, 1999; 4 = Spurgeon et al., 2000.

4.7. Internal metal concentrations relative to effect concentrations

Table 7 shows internal concentrations of heavy metals in earthworms reported in the literature for species showing (sub)lethal effects. Data were only used if it was clear which metal species was the cause of effects.

Comparing metal concentrations in *Lumbricus rubellus* in LH with data in Table 7 shows that Zn and Cu concentrations are higher or close to concentrations in earthworms showing effects on survival, cocoon production and growth during one or two sampling periods. During one sampling period, Zn concentrations in *Lumbricus rubellus* from PO were higher than concentrations in earthworms showing effects on cocoon production and survival. Zn concentrations in *Aporrectodea caliginosa* from PO were higher than concentrations in earthworms showing effects on survival.

4.8. The usefulness of internal effect concentrations

The assumptions on which the use of internal effect concentrations as indicators of effects of heavy metals are based and its advantages and disadvantages are discussed by Van Straalen (1996). The existance of a clear relation between whole body concentrations of heavy metals and effects of heavy metals is an important assumption. However, Spurgeon and Hopkin (1996b) showed that survival and cocoon production of *Eisenia fetida* were not related to internal Zn concentrations. Concentrations of the essential metals Zn and Cu, found in animals significantly affected by heavy metals (Table 7), are sometimes close to or within the range of internal reference concentrations shown in Table 6. Spurgeon et al. (2000) reported higher Zn concentrations in *Aporrectodea caliginosa,* showing no significantly reduced survival, than in worms showing significant effects on survival. Spurgeon and Hopkin (1999) reported a significant effect on survival of *Lumbricus rubellus* from a reference population at lower internal metal concentrations than from a polluted site. Based on these studies, it can be questioned if there is a clear relation between whole body concentrations of heavy metals and effects of heavy metals.

Data shown in Table 7 are based on internal concentrations of individuals at the end of toxicity experiments conducted to study effects on (sub)lethal endpoints. It is possible that effects were the result of a decrease in consumption and assimilation of energy and not due to critical metal concentrations in tissues.

5. Conclusion

The combination of high total soil concentrations of heavy metals and low pore water and plant leaf concentrations shows that risk assessment for isopods, millipedes and earthworms in floodplain soils should not be based on total soil concentrations only. Because the contribution of possible exposure routes to the uptake of heavy metals by detritivores is difficult to determine in the field, taking internal metal concentrations into account is useful. However, as a result of the large number of factors influencing internal metal concentrations, large standard deviations and possible adaptation mechanisms, comparison of internal metal concentrations between areas is difficult. More research is needed to see if ranges of internal metal concentrations can be defined that correspond to specific effects, independent of the large number of confounding factors. Bearing in mind these uncertainties, this study shows that in floodplain soils in the Biesbosch, the Netherlands, isopods are not affected by heavy metal pollution, millipedes are affected by Cu and earthworms are affected by Cu and Cd and possibly by Zn and Pb, if assimilation from ingested soil particles is significantly contributing to exposure.

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