CRITICAL METAL CONCENTRATIONS FOR FOREST SOIL INVERTEBRATES

A Review of the Limitations

GÖRAN BENGTSSON and LENA TRANVIK

Department of Ecology, University of Lund, Helgonavägen 5, S-223 62 Lund, Sweden

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Abstract. Based on a review of the literature on metal effects on forest soil invertebrates 100 to 200 mg Pb, <100 mg Cu, <500 mg Zn and 10 to 50 mg Cd kg^{-1} soil or litter are suggested as maximum allowable metal concentrations that will cause no adverse effects. These critical levels, emphasizing effects on abundance, diversity and life history parameters, are considered conservative and tentative, since few of the surveys they rely on were designed for critical level assessment, especially with respect to long-term consequences on decomposition and nutrient regeneration. The variation between animal species and individuals in susceptibility to metals due to differences in uptake, storage and tolerance are addressed.

1. Introduction

This review is primarily concerned with evidence from field site studies for effects of metal pollution on species abundances and diversity of soil animals. Such information is confined to a few surveys of some major emission sources, mainly in N. America and Europe. The first section of the paper summarizes metal effects on soil fauna population and 'community' characteristics in the vicinity of smelters. In later sections field data are analyzed with reference to different experiments on metal distribution in animals, life history parameters and tolerance and adaptation, and efforts are made to reveal mechanisms and processes underlying population and community changes in the field. The final section addresses the consequences of affected populations, as demonstrated by effects on decomposition.

2. Metal Effects on Species Abundance, Distribution and Diversity

There are considerable differences in the susceptibility to metals between different taxonomic groups, as demonstrated in a study of the soil litter fauna near a large primary zinc, lead and cadmium smelter at Avonmouth near Bristol in SW England, (Hopkin *et al.* 1985). Millipedes and earthworms, for example, were greatly reduced in numbers, whereas e.g. centipedes and large woodlice were apparently unaffected. Springtails, mites, dipteran larvae and predatory beetles increased in numbers per unit area (Table I).

The numbers of individuals and species of carabid beetles at Avonmouth were not significantly correlated with metal concentrations, whereas the species diversity was (Read *et al.*, 1987). From a study of a number of polluted forests Lesniak

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TABLE I

Density of major invertebrate groups (number of individuals m^{-2}) in leaf litter and soil from a contaminated woodland 3 km downwind of a smelting works (Hallen) and a simular but uncontaminated site (Wetmoor) at Avonmouth, England. From Hopkin *et al.* (1985)

Litter standing crop (kg m^{-2} dry wt)		Hallen 14.28	Wetmoor 1.35
Isopoda	*Oniscus asellus* (Linnaeus)	56	20
	Trichoniscus pusilkus (Brandt)	0	151
Diplopoda	Polydesmidae	8	79
	Julidae	0	11
	Glomeridae	0	21
Chilopoda	Lithobiidae	112	116
	Geophilomorpha	328	263
Arachnida	Acari	129000	19400
	Araneae	248	81
	Pseudoscorpionidae	200	67
Insecta	Collembola	20800	8688
	Coleoptera	902	48
	Coleoptera (larvae)	120	4
	Diptera (larvae)	4590	291
Annelida	*Lumbricus rubellus* (Hoffmeister)	17	3
	Lumbricus terrestris (Linnaeus)	0	29
	Allolobophora longa (Ude)	0	4
	Allolobophora caliginosa (Savigny)	0	30
	Octolasium cyaneum (Savigny)	0	9

(1980) was able to confirm the pattern of a reduction in the proportions of rare species and an increase in the proportion of common carabid species. In a mixed oak forest at Palmerton, Pennsylvania, Strojan (1975) performed pitfall trapping and found that the macrofauna responded in the same way as the microarthropods to metal contamination. Among the groups found Carabidae had the greatest decline in numbers.

In the vicinity of a brass mill at Gusum, SE Sweden, the carabid beetle community within 300 m from the mill was found to be less diverse than corresponding communities at sites further away (Bengtsson and Rundgren, 1984). At a boundary zone about 1 km from the mill the total species number peaked (Figure 1), and carnivore groups such as carabid beetles and harvestmen were the major contributors to the increase of species number. Herbivore groups, such as ants and weevils, seemed to be less susceptible to metals and showed an overall less response to the contamination. Hence, the total species numbers were clearly reduced at sites containing more than 2500 mg Cu kg^{-1} and 3600 mg Zn kg^{-1} in the litter layer.

In Palmerton oak forest Strojan (1978a) also found that the total arthropod density at a site 1 km from a zinc smelter, with 340 mg Cu kg^{-1} and 25 750 mg Zn kg^{-1} litter, was only 22% of that at the control site 40 km away. Six km from

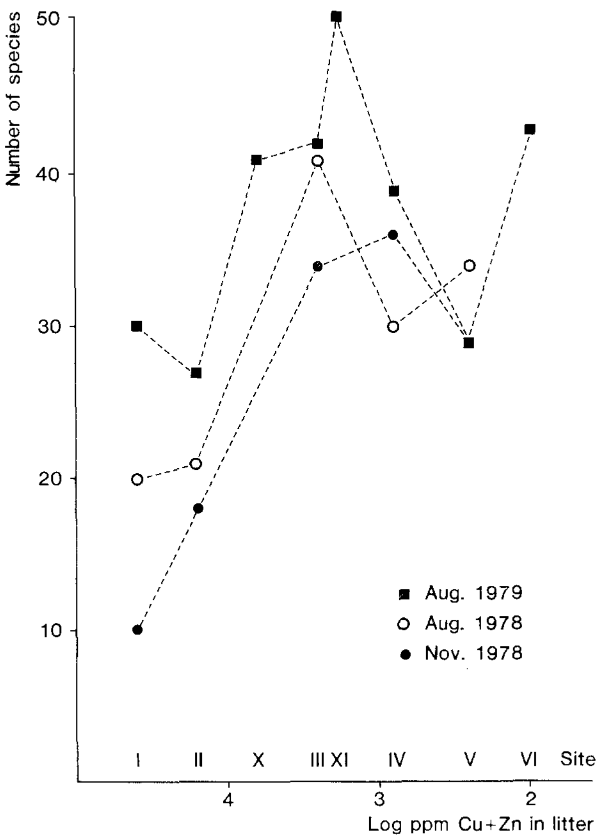


Fig. 1. Total number of species of spiders, harvestmen, ants, slugs and beetles trapped at the study sites at Gusum, Sweden, in relation to the concentration of Cu+Zn (from Bengtsson and Rundgren, 1984).

the smelter (172 mg Cu kg^{-1} and 14 600 mg Zn kg^{-1} litter) the density was 45% of that at the control (Figure 2). Oribatid mites showed the greatest absolute decrease, whereas Collembola seemed least affected by the soil metal concentration. The relatively small effect on Collembola as a group was suggested to be due to their high tolerance to metals compared to other arthropods or just an increase in one of several metal tolerant species of Collembola. Joosse and Buker (1979) observed various detoxifying mechanisms in Collembola, whose survival was high even when they were exposed to abnormally high doses of Pb. The relative amount of Cu accumulating in Collembola at the most contaminated site at Gusum was lower than at other sites suggesting an adaptation to metals (Bengtsson and Rundgren, 1988), and van Straalen *et al.* (1987) demonstrated enhanced Pb and Cd excretion efficiencies in Collembola from a contaminated site.

A large survey of the impact of metals on community characteristics of Collembola was performed in the Gusum area (Bengtsson and Rundgren, 1988). Neither species numbers, density nor species diversity were linearly correlated with metal concen-

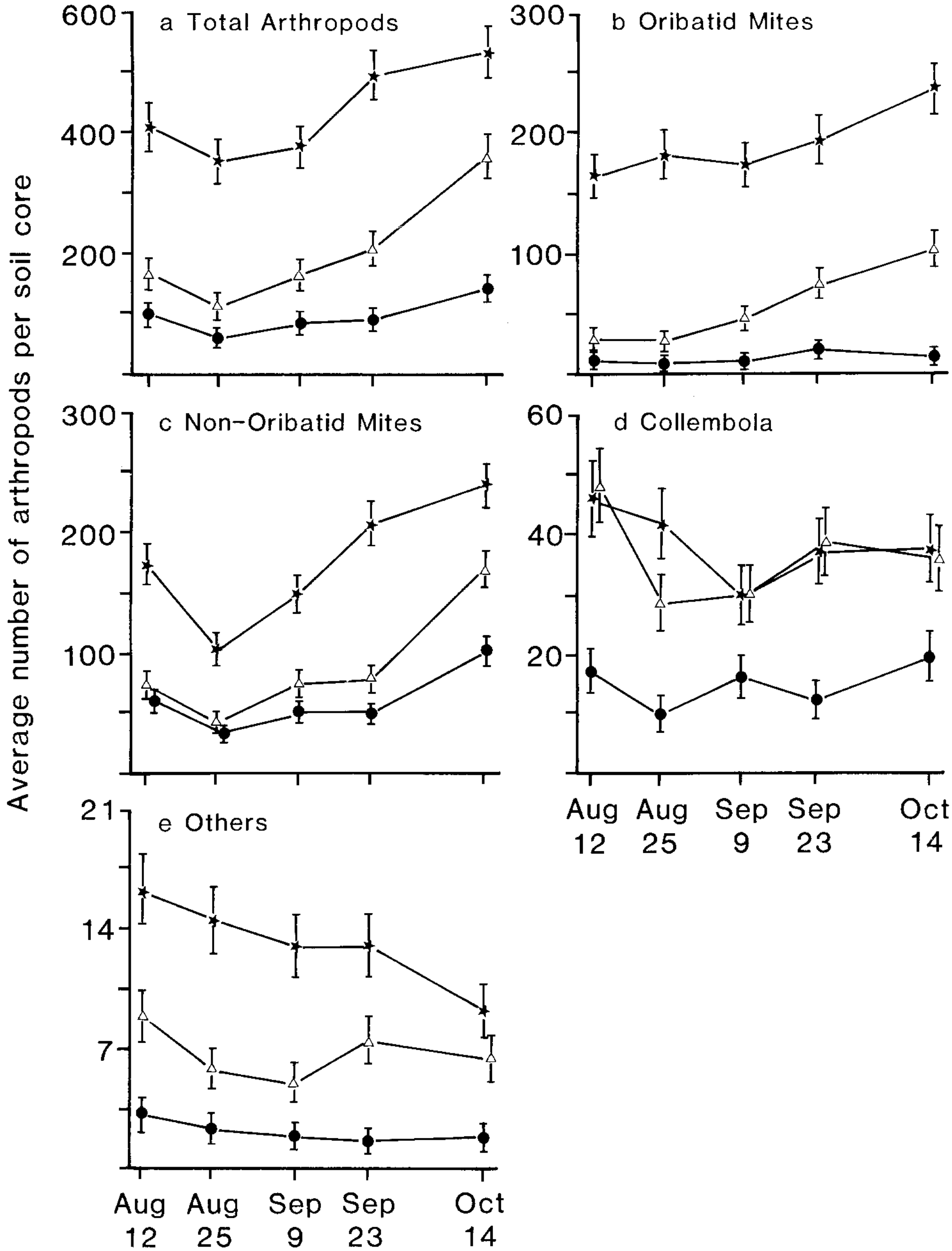


Fig. 2. Average number of soil arthropods per sampling core at 40 km (○), 6 km (□) and 1 km distance (●) from a zinc smelter at Palmerton, Pennsylvania, USA (from Strojan, 1978a).

trations but showed a bell shaped distribution with a peak at intermediate metal concentrations. The vertical distribution was clearly dependent on soil metal concentrations, and the densities at 2 to 10 cm depth were positively correlated with metal concentrations at 0 to 2 cm depth (Figure 3). The collembolan community at the most polluted sites had the lowest diversity indices, which was associated with a predominance of one species, *Folsomia fimetarioides.*

Information about the impact of metals on enchytraeids and nematodes is sparse. Emissions of mainly Cu and Zn reduced the density of enchytraeids and restricted their vertical distribution in the Gusum area (Bengtsson and Rundgren, 1982). Biomass, diversity and number of species in nematodes decreased along with increasing Pb concentrations in an industrial area in the river Po plain, N Italy (Figure 4) (Zullini and Peretti, 1986). Reduced diversity is a common response in a community under pollution stress (Culliney *et al.*, 1986) and primarily involves elimination of the rare and more sensitive species. In Nematoda, the suborder Dorylaimina was found to be most sensitive to Pb and its contribution to the nematode community at a concentration of >300 mg Pb kg^{-1} was very low (Figure 4). Bisessar (1982) found a similar negative correlation between levels of Pb, As, Cd and Cu and population density of nematodes near a secondary lead smelter in Ontario, Canada, (Table II).

Earthworms were also negatively affected by the emissions at Gusum (Bengtsson *et al.*, 1983b). Their densities and biomasses increased with decreasing concentrations of metals in the litter (Figure 5), and a higher juvenile mortality (or reduced fertility) was indicated at the most polluted sites compared with the control sites. As litter and humus are the principal metal sinks in the soil, surface living earthworm species run the highest risk of extinction. This was demonstrated at one of the most polluted sites (at 275 m distance), where the deep burrowing species *Allolobophora caliginosa* was the only earthworm representative. *Dendrobaena octaedra* was a common inhabitant of the litter and mor layers at Gusum except at sites with a litter Zn+Cu

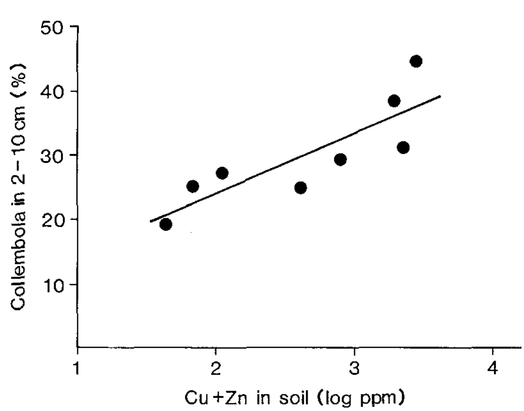


Fig. 3. Relative abundance of Collembola at 2-10 cm soil depth as a function of Cu+Zn concentrations at 0-2 cm soil depth (from Bengtsson and Rundgren, 1988).

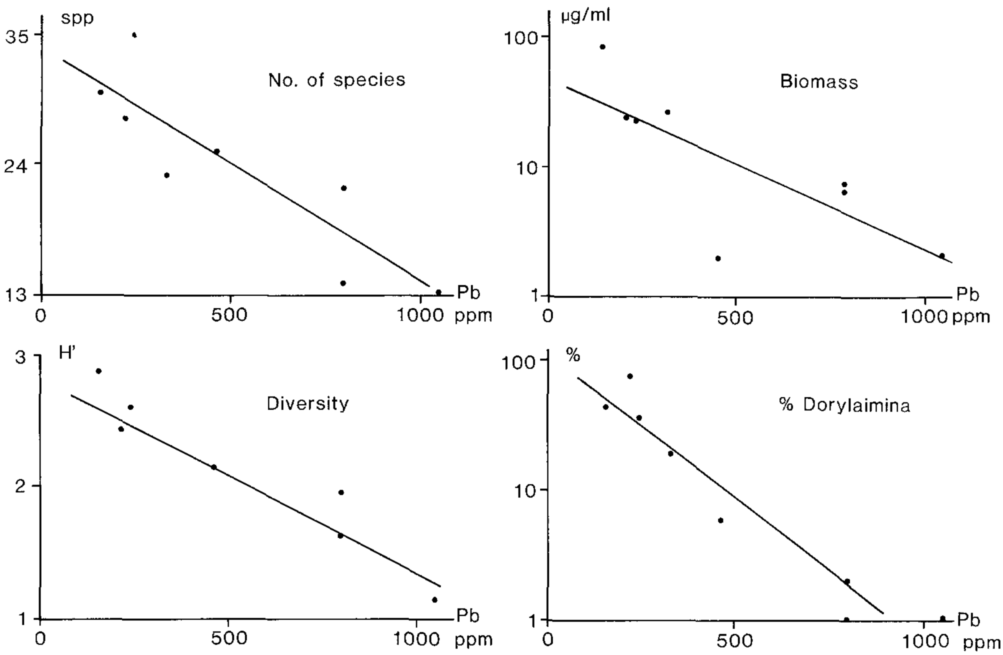


Fig. 4. Number of species, biomass, Shannon diversity index of nematodes, and percentage of *Dorylaimina* in stations with different Pb content in an industrial area in Italy (from Zullini and Peretti, 1986).

concentration of >6000 mg kg^{-1}. About 35 000 mg Zn+Cu kg^{-1} litter was lethal to earthworms of all species. No earthworms were found in soils with a concentration of 5300 mg Pb+Cu+As+Cd kg^{-1} soil (average of 0 to 10 cm depth) or more at the Pb smelter in Ontario (Table II) (Bisessar, 1982). Their numbers were halved at about 4000 mg Pb+Cu+As+Cd kg^{-1}, probably as a consequence of a more than 400% increase of Pb and Cd concentrations. The remaining 50% of the earthworm population disappeared as Pb, As and Cd concentrations increased by another 25 to 40%. As a comparison, the average concentration of Zn+Cu of 0 to 10 cm depth at the aforementioned site at Gusum was 515 mg kg^{-1} soil. Cu concentrations of 40 mg kg^{-1} alone may reduce the number of earthworms, as shown by Streit (1984) in a field simulating experiment with *Octolacium cyaneum*.

Wright and Stringer (1980) compared densities of earthworm populations at 4 and 9 km distances from the zinc smelter at Avonmouth. They found no evidence for a reduced population density at the 4 km site, though the Zn+Pb concentration in the soil was more than 300% higher there (764 mg kg^{-1}). The metal content of their contaminated site was less than 10% of that of the most contaminated site at Gusum (Table III). Regretably they did not study sites close to Avonmouth and were, thus, unable to establish whether earthworms were completely eradicated there like at Gusum. Williamson and Evans (1972) examined the population of invertebrates close to a major highway and found that the habitat change from

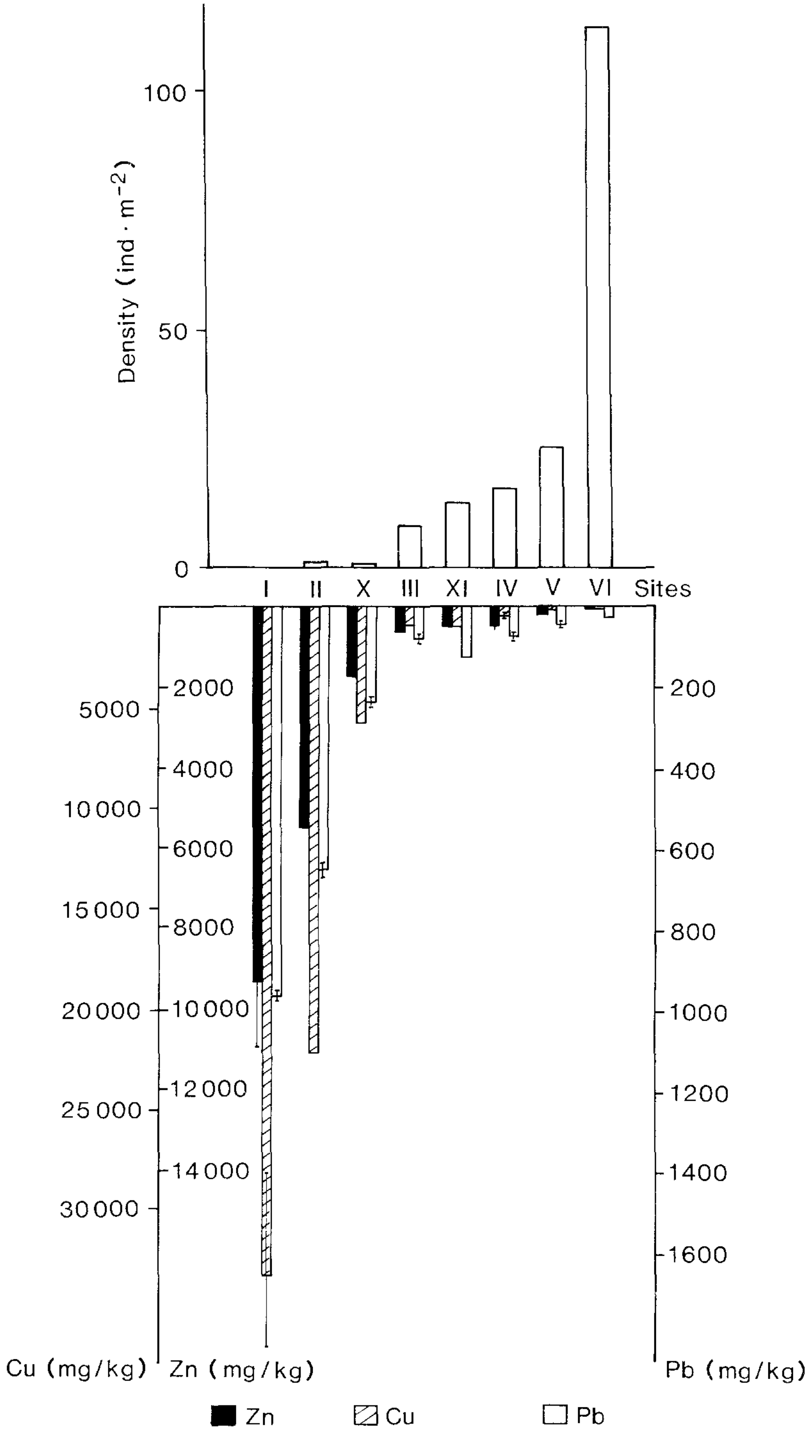


Fig. 5. Earthworm density and metal concentrations in litter in the Gusum area (compiled from Bengtsson *et al.*, 1983b and Bengtsson and Rundgren, 1984).

TABLE II

Metals (mg kg^{-1} dry wt) and soil fauna (numbers 10 g $soil^{-1}$) at four locations near a secondary lead smelter in Ontario, Canada. From Bisessar (1982)

Location east of source	Metals				Soil fauna	
	Pb	As	Cd	Cu	Nematodes	Earthworms
15 m (site 1)	28000	972	151	599	16.0	0
90 m	8333	554	102	398	30.9	0
150 m	4800	230	33	287	26.6	0
180 m	3564	163	26	333	58.0	1.3
Control (1000 m south)	703	57	5	73	98.0	2.3

TABLE III

Average metal concentration (mg kg^{-1} d.w.) in litter and soil from two sites in the Avonmouth area, England, and four sites in the Gusum area, Sweden

Metals	Pb	Zn	Cd	Cu
Sites and distances from emission source				
Long Ashton 9 km[a]	92	89	1	nm
Severnside 4 km[a]	147	617	10	nm
Gusum 7.8 km[b]	18/44/48	40/36/233	nm	5/7/9
Gusum 2.9 km[b]	20/40/94	35/83/770	nm	9/27/231
Gusum 275 m[b]	14/27/637	160/1680/10952	nm	9/214/9095
Gusum 175 m[b]	34/71/990	184/2222/20208	nm	25/533/16500

[a] 0–10 cm depth in soil; from Wright and Stringer (1980).
[b] 8–10 cm depth in soil (first number), 0–2 cm (second number), litter (third number); from Bengtsson *et al.* (1983b).
nm = not measured.

roadside verge to pasture field affected the abundance more than the variation in Pb. Also here the highest level of Pb in the soil was about 10% of that of sites in the vicinity of the brass mill at Gusum.

A summary of the susceptibility of some soil invertebrate groups and some structural variables at the described metal polluted field sites is given in Table IV. Figures of the lowest metal concentrations observed to affect soil animals in the field were derived from conclusions drawn in the cited literature and from comparisons by t-tests of means of abundances at the field sites at Gusum. The lowest concentrations at which changes were observed in the field are certainly

TABLE IV

Effects of metals on field performance of soil invertebrates; abundance, diversity, distribution, and biomass. [1]0-2 cm soil depth; [2]litter

Group	Lowest metal concentration (mg kg^{-1}) in soil and litter with a significant ($p<0.05$) effect				Affected parameter	Source
	Pb	Zn	Cu	Cd		
Nemtodes	200				biomass	Zullini and Peretti (1986)
	300				diversity index	Zullini and Peretti (1986)
	300				species number	Zullini and Peretti (1986)
	3564		333	26	density	Bisessar (1982)
Enchytraeids[1]	36	171	78		density	Bengtsson and Rundgren (1982)
	99	2023	474		vert. distribution	Bengtsson and Rundgren (1982)
	34	2068	212		species number	Bengtsson and Rundgren (1982)
Earthworms[1]	36	171	78		density	Bengtsson *et al.* (1983b)
	99	2023	474		biomass	Bengtsson *et al.* (1983b)
Earthworms	4800		287	33	density	Bisessar (1982)
			90		density	Streit (1984)
Collembola	2333	25750	340	885	density and	Strojan (1978a)
Mites	971	14600	172	256	diversity indices	Strojan (1978a)
Collembola[1]	74	649	126		vert. distribution	Bengtsson and Rundgren (1988)
Carabids	971	14600	172	256	species number	Strojan (1975)
Ants[2]	132	1165	657		abundance	Bengtsson and Rundgren (1984)
Spiders[2]	230	3585	2509		species abundance	Bengtsson and Rundgren (1984)
Total ground-living marcoinvert.		3600	2500		species number	Bengtsson and Rundgren (1984)

different from those concentrations for which changes may be observed, for a number of obvious reasons.

One such is the sampling frequency in relation to the variance around the sample mean. If the number of replicates is sufficiently large, even very small changes may be observed with a certain probability, as discussed elsewhere (Bengtsson and Rundgren, 1982, Bengtsson *et al.*, 1988). Whether such changes are sufficient to influence the fate of species or populations can hardly be predicted without accurate knowledge about population fluctuations, life history parameters, tolerance, metal allocation, etc. Small changes in population density, whether observed directly in the field by simulation experiments or predicted from observations of e.g. reproduction and mortality in laboratory experiments, may be outweighed by immigration and adaptation, so that a documentation of long-term changes of the soil biota in metal polluted environments becomes a formidable task. The establishment of critical concentration values would then require a careful risk analysis based on the limited data on metal assimilation and accumulation, life history parameter changes and consequences of metals on e.g. decomposition.

Another reason is the practice to use exponential rather than log-normal frequency distribution of sampling in pollutant gradients, so that sites with very severe pollution become overrepresented. This practice, motivated by the concern about the combination of labour intensive observation technique, a substantial between-sample variation, and short grant periods, was efficient to demonstrate effects, if any, of metals but unable to support sufficient data for an analysis of critical levels.

A third shortcoming depends on the complex field situations examined. Different combinations of metals at enhanced concentrations in soil and litter and differences in pH and organic carbon content make comparisons of metal effects between different surveys and even between different sites in the same area difficult. Very few sites are polluted exclusively by one metal, and the known and reported effects on soil animals are in most cases due to unique combinations of metal species and soil characteristics, such as pH and binding sites for metals. Though the relative toxicities of metals may be extracted from a textbook, comparisons site by site will be hampered by interactive phenomena, tending to introduce more than just additive effects at certain metal concentrations or less than additive effects due to e.g. competition between metals for binding sites in animal tissues.

In conclusion, a common effect of metal contamination in soil animal groups is a decrease in species diversity. In some groups, such as microarthropods (excluding oribatid mites) and carabid beetles, the total abundance is not clearly affected, as the decrease in some species is compensated for by an increased number of individuals of more tolerant species. Soft-bodied animals such as earthworms seems to be more directly affected by the enhanced metal concentrations and a reduced species diversity is accompanied by a decreased density and biomass. Whereas earthworm and enchytraeid densities are reduced in soil with a Cu concentration of less than 100 mg kg^{-1} (and other metals at negligible concentrations, Table IV), the numbers of Collembola remain the same even at a concentration twice as high.

3. Uptake, Accumulation and Compartmentalization of Metals in the Organism

Some of the variation in effects on species abundance and diversities may be explained from differences in uptake and accumulation of metals, which has been shown by analysis of field collected animals and in short-term exposure experiments in the laboratory. To our knowledge the only work where performance of invertebrates in the forest soil and metal concentrations in their body tissues have been simultaneously measured is that reported from the Gusum area. By recombining data supported by Bengtsson *et al.* (1983b) and Bengtsson and Rundgren (1984, 1988) the statement that the distribution of soil animals is directly limited by toxic metal concentrations can be examined from correlations between body Cu concentrations and abundance/density numbers.

With one exception, a negative correlation was found between the concentration of Cu in body tissues and the density/abundance of some representative species (Figure 6). The data base is generally weak, with the slope of the correlation in many cases determined by observations at one field site with much lower density and higher Cu concentration than the other sites. If we accept the correlations, with some reservations, we find:

(i) that the tissue Cu concentrations were not significantly raised until population density was halved in comparison with the maximum density observed; or, alternatively, that changes in population densities were directly proportional to changes in tissue metal concentrations;

(ii) that soft-bodied predators such as the spider *Trochosa terricola* had considerably higher Cu concentrations than hard-bodied predators such as the carabid beetles *Pterostichus niger* and *Pt. melanarius* and;

(iii) that cerebral ganglion of earthworms was a better Cu tracer than muscles.

From the graphs (Figure 6) we may tentatively suggest that body concentrations above 70 $\mu g\ g^{-1}$ of Cu reduce the abundance of *A. caliginosa*. Similarly, 140, 50, and 100 $\mu g\ g^{-1}$ are associated with toxic levels for *D. octaedra*, carabids and *O. armatus*, respectively. Bengtsson *et al.* (1986) observed significant reduction of cocoon production by the earthworm *Dendrobaena rubida* at 294 $\mu g\ g^{-1}$ Cu in ganglion, which was associated with the lowest Cu treatment used for a number of experimental soils. Thus, cocoon production may be reduced at concentrations that are closer to the level where the abundance of *D. octaedra* is affected.

The correlation between body concentration of Cu and density of the collembolan species *Onychiurus armatus* was exceptional (Figure 6e); the Cu concentration was lowest at sites where the population density was lowest, and the highest concentrations were found in specimens from populations with intermediate densities. This pattern was partly a consequence of tolerance evolved by individuals under the most severe metal conditions, as indicated by their low body concentration of Cu in relation to the concentration in soil (Bengtsson and Rundgren, 1988). Other variables, such as predation, parasitism, niche competition and unfavorable microclimate, may reduce population density in unpolluted environments considerably more than metals would do in polluted environments; in such cases laboratory studies on uptake, accumulation and susceptibility to metals are little help to explain the differences in correlation patterns between species. In yet other cases such experiments will give a more accurate and precise judgement of dose-response relationships.

The body concentration of metals in invertebrates, as evidenced from the literature, differs between taxonomic groups, species and even individuals within one species. This was illustratred by Hunter *et al.* (1987), who ranked groups of invertebrates collected in the vicinity of a Cu/Cd plant on basis of their concentration of Cd in decreasing order as follows: Isopoda, Oligochaeta, Arachnida, Collembola, Carabidae, and Chilopoda. The corresponding order for Cu was: Isopoda, Collembola, Oligochaeta, Arachnida, Chilopoda, and Carabidae. The outstanding capacity of Isopoda to accumulate metals was noticed in the 1960's by Wieser (1961), who collected *Porcellio scaber* from disused mine sites and found that their hepatopancreas had the highest ever recorded Cu concentrations in soft tissues of any animal. In general, soil invertebrates possessing hemocyanin as respiratory pigment, such as isopods, gastropods and spiders, tend to have much higher Cu concentrations than other groups (Wieser, 1979).

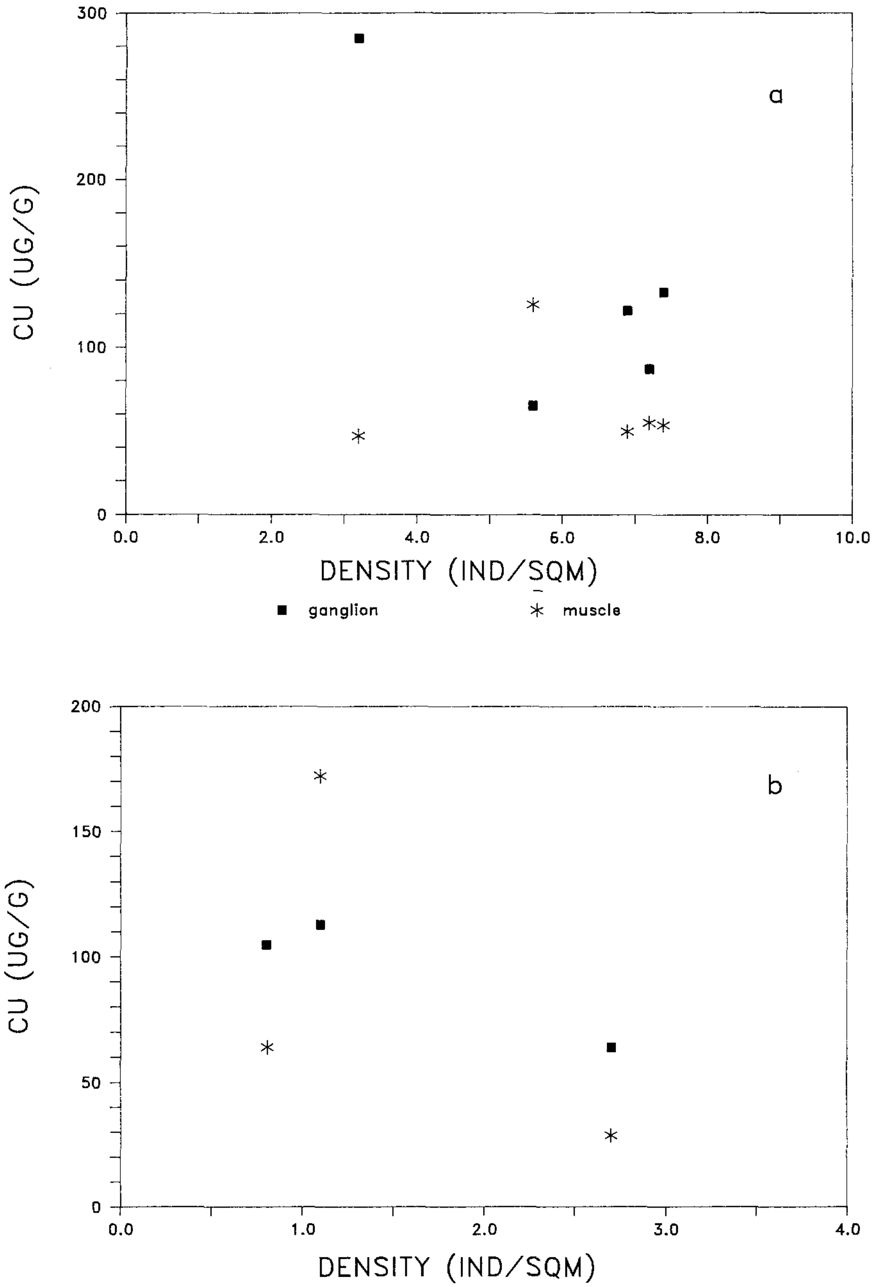
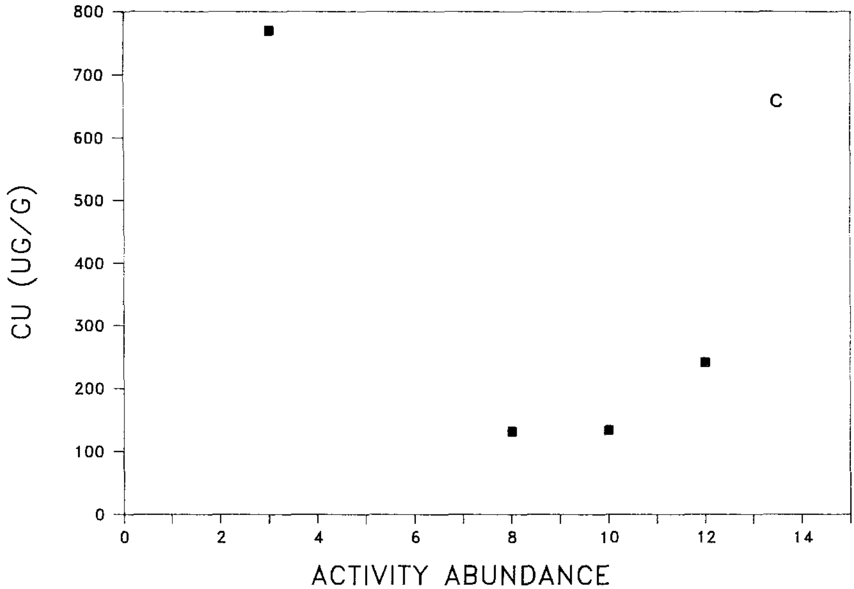
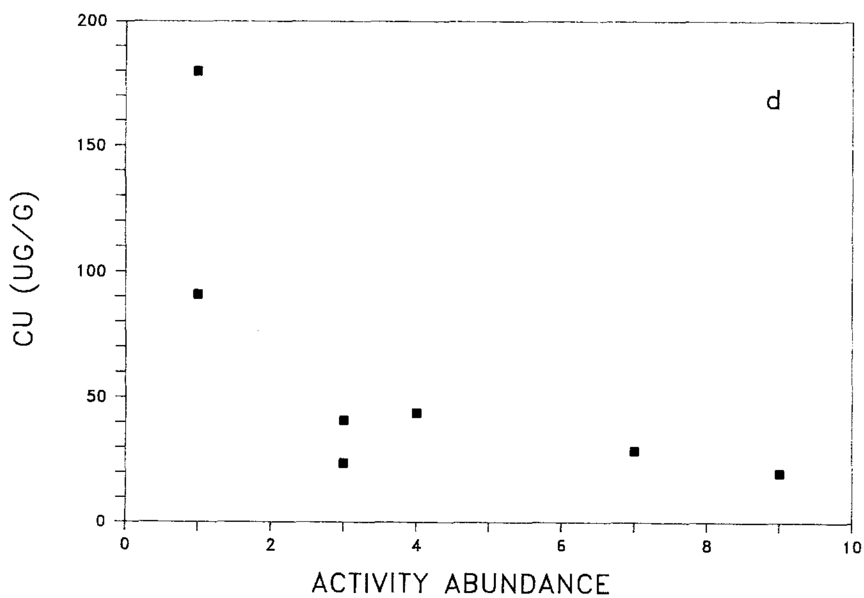
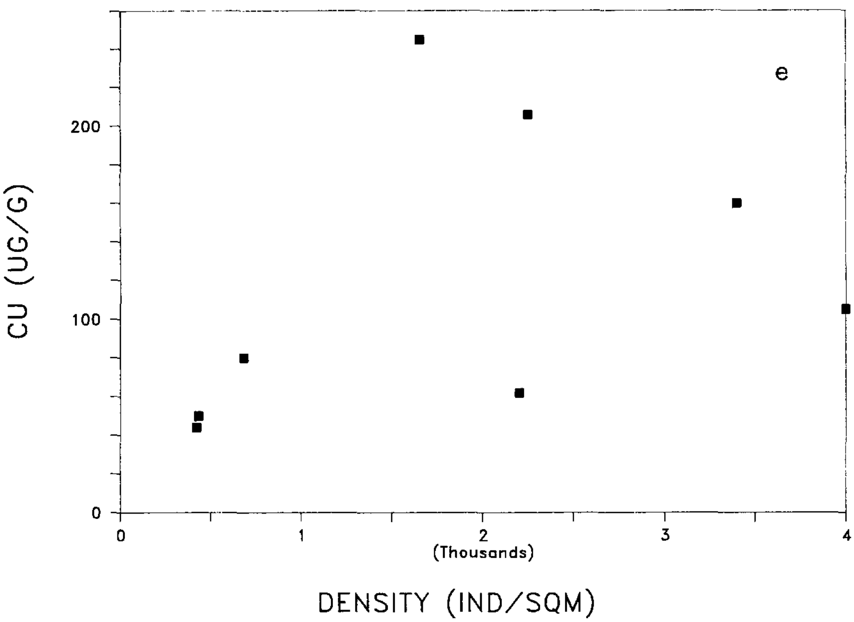


Fig. 6. Correlations between density or activity abundance of soil invertebrates in the field and metal concentrations in their tissues; (a) *Dendrobaena octaedra* (ad.), (b) *Allolobophora caliginosa* (juv.), (c) *Trochosa terricola*, (d) carabids, (e) *Onychiurus armatus*.





Efforts to find general patterns in metal distribution describing concentration as a function of body size (van Straalen and van Wemsen, 1986) or trophic level (Price *et al.*, 1974; Roberts and Johnson, 1978) have been unsuccessful, and ecological generalizations to predict the rate and degree with which species or individuals accumulate metals may require a more thorough understanding of similarities between species in physiological mechanisms of metal distribution. The body content



is determined by many factors, some of which (e.g. soil concentration, availability of metals, intrinsic rate of bioaccumulation and tolerance of the organism) seem to be more important than others (Ma, 1982). In addition, Coughtrey and Martin (1977) emphasized age, species physiology and general condition in the degree of metal accumulation in an individual.

Three main biological factors control metal accumulation in different groups of terrestrial invertebrates: 1) the diet, 2) the structure and physiology of the digestive system and 3) the mechanisms by which metals are stored within cells in an insoluble form.

The diet

Susceptibility to metals in some species might be a secondary effect due to differences in food availability and quality rather than metal toxicity *per se*. Spiders usually have higher metal concentrations than both carabid beetles and centipedes (Bengtsson and Rundgren, 1984, van Straalen and van Wemsen, 1986, Hunter *et al.*, 1987), which is thought to be related to their feeding strategy and digestive system rather than to an active uptake of some metals as was suggested for isopods. The extent to which carnivorous invertebrates assimilate metals depends on the choice of prey species and also on what parts of the prey that are eaten (Hopkin, 1986). Spiders of the genus *Dysdera*, which feed on terrestrial isopods, suck out tissues including hepatopancreas – the major metal storage in isopods (Hopkin and Martin, 1984a) – where metals are more concentrated than in the whole woodlice. The surplus of metals from such selective feeding is embedded in intracellular granules formed in the midgut and excreted by lysis of the cell. Large numbers of these granules have been observed in spider faeces (Hopkin, 1986).

Lithobiid centipedes have a simpler digestive system than the *Dysdera* and lack midgut diverticulae. When fed on the hepatopancreas of isopods they are unable to digest the metal containing granules of the woodlice, which are voided in the faeces, apparently unchanged (Hopkin, 1986). The spider, *Centromerus sylvaticus*, which is specialized on collembolan prey (Ernsting and Joosse, 1974), was found to have ten times higher body concentrations of metals than the carabid beetle *Notiophilus biguttatus* when they preyed the same test population of Collembola (van Straalen and van Wemsen, 1986). The difference was suggested to be a consequence of the spiders sucking out their prey with little defaecation and the carabids swallowing the whole prey and producing significant amounts of faeces.

Also the life stage of the prey determines the metal dose which the predator is exposed to. *N. biguttatus* was demonstrated to have a preference for small prey of the Collembola *Orchesella cincta* (Ernsting and Mulder, 1981), which implies that its exposure to metals could be lower than would be deduced from the average concentration of the *Orchesella* population (van Straalen *et al.*, 1986).

Effects of environmental pollution on earthworms have been studied for the last 20 yr, and earthworms have been sampled in various habitats, such as roadside soils (Williamson and Evans, 1972; Gish and Christensen, 1973; Ash and Lee, 1980),

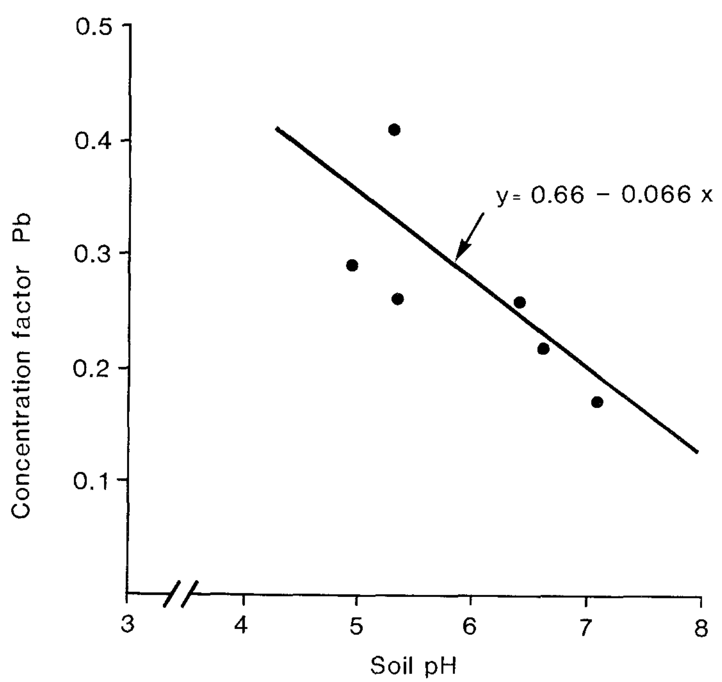


Fig. 7. Mean Pb concentration factors in *Lumbricus terrestris* against soil pH (from Morris and Morgan, 1986).

mines and industrial areas (Ireland, 1975, 1979; Ireland and Richards, 1977; Wright and Stringer, 1980; Bengtsson *et al.*, 1983b) and analysed for metal concentrations. In the early studies accumulation of metals was often related directly to soil metal concentration (Van Hook, 1974; Czarnowska and Jopkiewicz, 1978), but in later work the relations between soil pH, organic matter, and soil moisture on one hand and metal concentrations in soils and earthworms on the other have been examined. The influence of pH on metal uptake has recently been of major concern, and an inverse relationship has been demonstrated for *Lumbricus rubellus, Allolobophora caliginosa* and *L. terrestris* (Ma, 1982; Morgan, 1985; Ma *et al.*, 1983; Morris and Morgan, 1986) (Figure 7).

When all abiotic factors are kept constant, each metal shows a characteristic dose-response relationship (Figure 8), and the ratio of the concentration of a specific metal in the worm body to that in the soil (concentration factor) generally varies inversely with the degree of soil contamination (Ma, 1982; Ma *et al.*, 1983) and metals species. In earthworms collected at roadsides the concentration factors of Cd, Ni, Pb and Zn averaged 11.2, 1.9, 0.95, and 5.7 (Gish and Christensen, 1973). Also Morgan (1985) calculated a concentration factor below 1 for Pb, presumably reflecting the high insolubility of this element in soils. Lead concentration in worms appears to closely follow that in soil, while the concentration factors of Zn, Cd and Cu decrease with increasing soil metal concentration (Ma, 1982; Morgan, 1985).

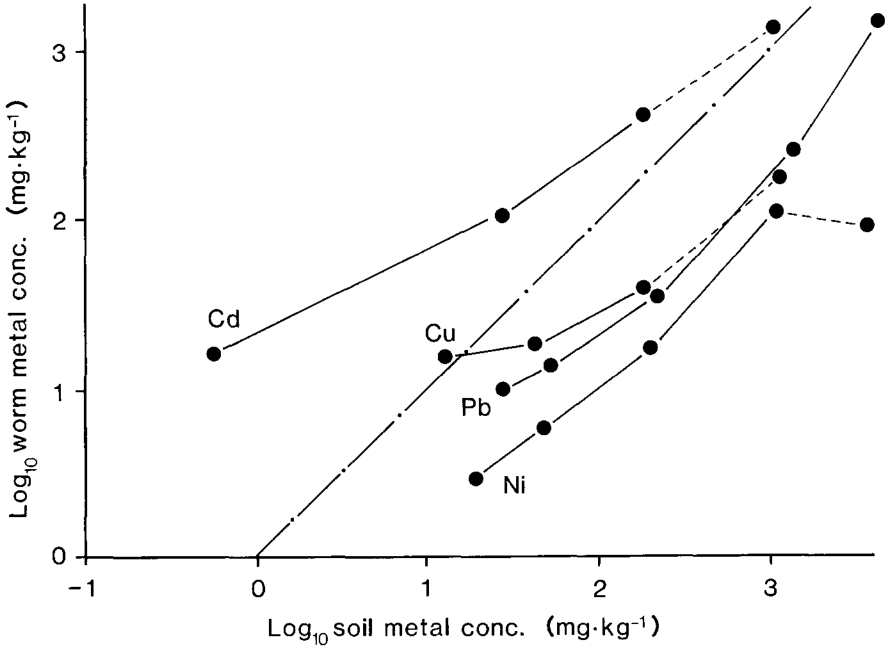


Fig. 8. Metal concentrations in adult *Lumbricus rubellus* after 12 weeks (——) or 6 weeks (--) exposure to sandy loam soil with added metal. The straight line indicates a hypothetical 1:1 relationship (from Ma, 1982).

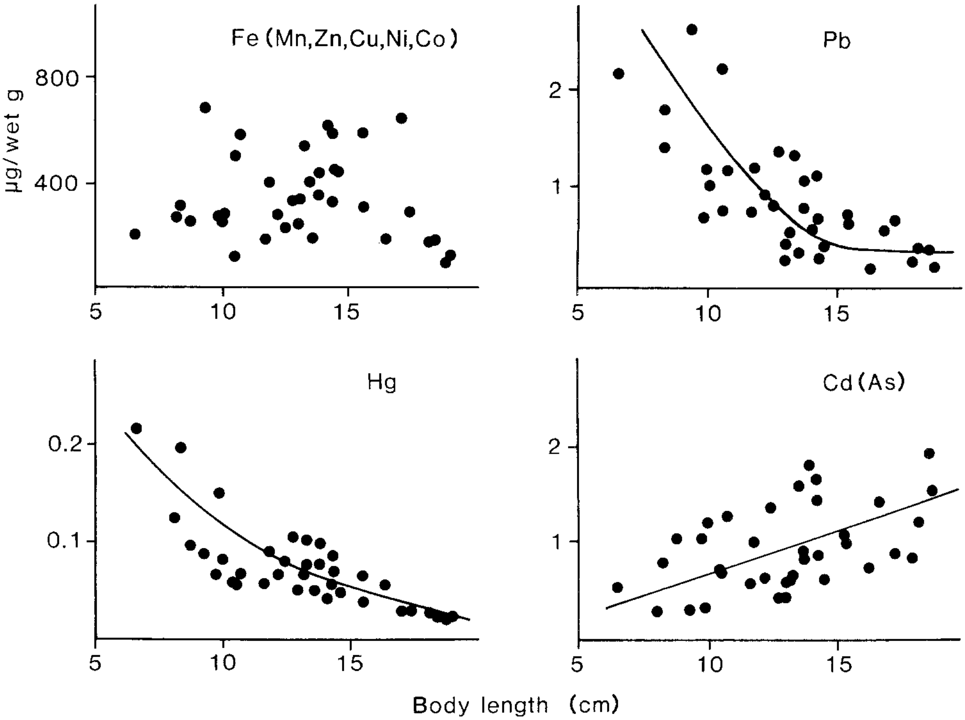


Fig. 9. Relations between body length of an earthworm, *Pheretima hilgendorfi* and its metal concentrations (from Honda *et al.*, 1984).

The body concentration of Cd in the earthworm *Pheretima hilgendorfi* increased with body length and weight whereas Pb concentrations decreased (Honda *et al.*, 1984) (Figure 9). The accumulation of Pb and Cd depends primarily upon the age or exposure time. A faster Pb saturation indicates that the biological half life of Pb in the earthworm is shorter than that of Cd. The concentrations of Zn, Cu and Ni varied widely with body length. The observations suggest that the accumulation of Zn, Cu, and Ni in the earthworms depends on metabolic turnover and that the metal concentration remains constant throughout the lifespan. Relationships between tissue metal concentration and body weight and age have also been observed in the snails *Helix aspersa* and *Cepea hortensis* (Coughtrey and Martin, 1977; Williamson, 1980). Boyden (1974) found that log-log transformation provides better descriptions of such relationships, whereas Williamson (1980) found that not even log-log plots always provide a good fit for data from the same sampling occasion. This and other anomalies indicate that changes in metal contents of molluscs may best be understood by considering the interaction of a variety of factors. Most of the unexplained variance is likely to be individual differences in recent diet and digestive activity (Williamson, 1980).

The digestive system

Non-essential elements may enter animals by the same biochemical pathways as essential elements, with which they are chemically related. Lead was suggested to be taken up along the same pathway as Ca (Beeby, 1978), and assimilation of Cd by the same biochemical routes as Cu and Zn (van Capelleveen, 1987a and references therein). The uptake of Pb in earthworms interacts with Ca, which is manifested at three ecological/physiological levels: 1) soil Ca tends to suppress Pb accumulation (Andersen, 1979, Ireland, 1979, Andersen and Laursen, 1982); 2) observed species differences in Pb body burdens may reflect fundamental differences in the Ca metabolism of the species involved (Ireland and Richards, 1977; Andersen and Laursen, 1982; Morgan and Morris, 1982); 3) tissue Pb and Ca accumulations are paralleled (Ireland, 1974; Andersen, 1979). Pb and Ca are chemically very similar, and Pb often follow the same biochemical pathways as Ca so that an uptake is unavoidable in invertebrates with a high Ca demand. The statement that high soil Ca concentrations inhibits Pb accumulation in worms has been questioned, and the competitive role of soil Ca on Pb accumulation has not been unequivocally demonstrated (Morris and Morgan, 1986). A comparison between the concentration of Ca and Pb in whole bodies of three earthworm species revealed that both Ca concentration and the Ca: Pb ratio were significantly higher in *Lumbricus terrestris* than in *Allolobophora caliginosa* and *Octolasion lacteum*, whereas the Pb concentration was significantly lower. These species are fundamentally different in their Ca metabolism. As *A. calignosa* and *O. lacteum* lack secretory calciferous glands (Piearce, 1972), less Ca is transported across their intestines.

Storage

Once exposed to metals by ingestion an animal may still escape their detrimental effects by storing them in one or more organs in a form which will not diffuse throughout the body and interfere with essential biochemical processes in other tissues (Hopkin and Martin, 1984a). The animal can also use efficient removal mechanisms, such as excretion by moulting and defecation (Joosse and Buker, 1979; van Straalen *et al.*, 1985).

In an experiment with the collembolan species *O. cincta* Joosse and Buker (1979) found that most of the Pb consumed was concentrated in the faeces. The assimilated Pb was mainly concentrated in the degenerated gut epithelium and was removed regularly through moulting. *Onychiurus armatus* fed with metal contaminated fungi was shown to concentrate metals during the first two weeks of its life cycle and then excrete metals towards a steady state level (Bengtsson *et al.*, 1983a). The periodic renewal of the gut epithelium at each moult is the main excretion mechanism in Collembola and prevents them from accumulating metals above the steady state.

The hepatopancreas is the most important storage organ of isopods for Zn, Cd, Pb and Cu (Hopkin and Martin, 1982a). At a contaminated site near a smelting work in SW England the hepatopancreas of woodlice constituted a mean of only

7% of the dry weight of the animals, whereas it contained a mean of 76% of the Zn, 95% of the Cd, 83% of the Pb and 85% of the Cu of the whole body (Hopkin and Martin, 1982a). In this experiment a significant positive correlation was found between the mean relative dry weight of the hepatopancreas of *Oniscus asellus* and the concentrations of Zn or Cd in leaf litter. It was suggested that animals from a heavily contaminated site have achieved tolerance to metals by increasing the binding capacity of the hepatopancreas. The enlarged hepatopancreas then enables them to detoxify a greater amount of metals.

Despite the high accumulation capacity for metals isopods are less susceptible to metals than many other groups due to the binding characteristics of the metals. In the hepatopancreas metals are stored in the epithelium within two types of cells, the B- and S-cells. Intracellular granules in these cells contain the metals; Zn, Cd, Pb and Cu are stored in granules within the S-cells, whereas the B-cells, which naturally contain Fe granules, also accumulate Zn and Pb (Hopkin and Martin, 1982b). Fine deposits of Zn and Pb may also be present on the membrane of the cells (Hopkin and Martin, 1982b) or scattered throughout the cytoplasm (Prosi *et al.*, 1983). The granules of the S-cells also contain large amounts of S. This indicates that metals are complexed with metallothionein proteins which contain the S rich amino acid cystein (Hopkin and Martin, 1984b and references therein). When the supply of proteins is exhausted the metals will turn into the intestinal wall without detoxification, and the animal will eventually die.

The concentration factors (ppm in animal: ppm in diet) of Zn and Pb are less than one tenth of those of Cd and Cu (Hopkin and Martin, 1984b). In experiments in which metal availability has been assessed, about 50% of the Zn, Cd and Cu have been shown to dissolve in chemical extractants similar to the digestive enzymes, whereas much smaller amounts of Pb were released (Martin *et al.*, 1976). This indicates that the low concentration factor of Pb is due to the low availability of this metal in the food. The difference between the assimilation rates of Zn, Cd and Cu are thought to be due to a lower efficiency of uptake of Zn (Hopkin and Martin, 1984b).

The metallothionein induction is, however, not a sufficient explanation for the extraordinary metal accumulation in isopods. A better explanation might be that Cu is essential for the oxygen carrying protein haemocyanin (Bonaventura and Bonaventura, 1980), and that the assimilation of this metal must be efficient. A drawback of the efficient Cu assimilation system is that Cd may be taken up along the same biochemical pathway in polluted soils and bind to Cu metallothioneins (Overnell and Trewella, 1979).

Metals concentrate selectively in the body tissues also in earthworms and seem to have high affinity for celebral ganglion and seminal vesicles (Bengtsson *et al.*, 1983b). Honda *et al.* (1984) found high concentrations of Zn in the preclitellar region where the reproductive organs and nervous and blood system are situated. The concentration of metals in cerebral ganglion and sarcoplasm may have profound effects on e.g. the soil turnover behavior of earthworms, while the concentration of metals in the seminal vesicles may affect their reproduction (Bengtsson *et al.*, 1983b). The postclitellar region with excretory and digestive organs, contained high concentrations of Cd and Cu. In other studies the gut and digestive system, especially the chloragog tissues, seemed to be the dominating site for metal accumulation (Andersen and Laursen, 1982; Ireland and Richards, 1981), which is suggested to interfere with glycogen metabolism in the absence of an energy rich food (Ireland and Richards, 1977).

The main site for storage and accumulation of metals in terrestrial molluscs is the digestive gland (Cooke *et al.*, 1979; Ireland, 1979, 1981; Coughtrey and Martin, 1977; Williamson, 1980). Cooke *et al.* (1979) calculated that up to 95% of the total Cd and Zn burden were contained therein. When fed artificially Cd polluted food, *Arion ater* was shown to accumulate Cd in the cytoplasmic fraction of the digestive gland cells (Ireland, 1981), where metallothioneins are normally found in molluscs. In *Helix aspersa* the metal binding protein was found to contain 6.0% S, which indicates that it is a metallothionein rich in the amino acid cysten, similar to that found in other animals. Both Zn and Cd form strong metal S bonds where the Cd-S bond is more stable than the Zn-S bond. A Zn binding protein appears to be naturally present in the body of *H. aspersa*, but Zn is partially replaced by Cd in polluted environments. In addition, Cd and Zn uptake induces the synthesis of extra metallothioneins which preferentially bind Cd (Cooke *et al.* 1979).

Seasonal and metereological variables complicate comparisons of ecological effects and body burdens of metals between sites and groups of animals. Daylength was shown to be a major determinant of the soft tissue metal concentration in the snail *Cepea hortensis* (Williamson, 1980), as the maximum rates of feeding and movement coincided with the period of maximum daylength. For *A. ater* the uptake of metals was significantly higher in July than in September (Ireland, 1981) which was thought to be due to their higher metabolic rate in July. High moisture conditions also enhance the activity of terrestrial molluscs, and consequently, metal content in *C. hortensis* peaked after humid periods, although prolonged heavy rains inhibited the activity and reduced the metal content (Williamson, 1980). Hunter *et al.* (1987) found that seasonal changes in the abundance, species composition and age structure of invertebrates caused substantial variation in body metal concentrations throughout the year (Figure 10) with the highest variation at the most polluted sites. They suggested that the use of annual mean metal concentrations seriously underestimated peak levels and thus exposure levels to predators feeding on soil invertebrates.

The rates of metal accumulation in uncontaminated or moderately contaminated sites were shown to be directly proportional to the growth rate in *O. asellus*, whereas at heavily contaminated sites the accumulation rate exceeded that of growth. The amounts of metals then increased exponentially until the detoxifying capacity was exceeded and the levels in the blood increased to toxic levels and animals died (Hopkin and Martin, 1984b). Joosse *et al.* (1984) found that a tolerant population of the isopod *Porcellio scaber* from a contaminated site, regulated its body content of Zn at a higher level than a control population. Both increased their body content

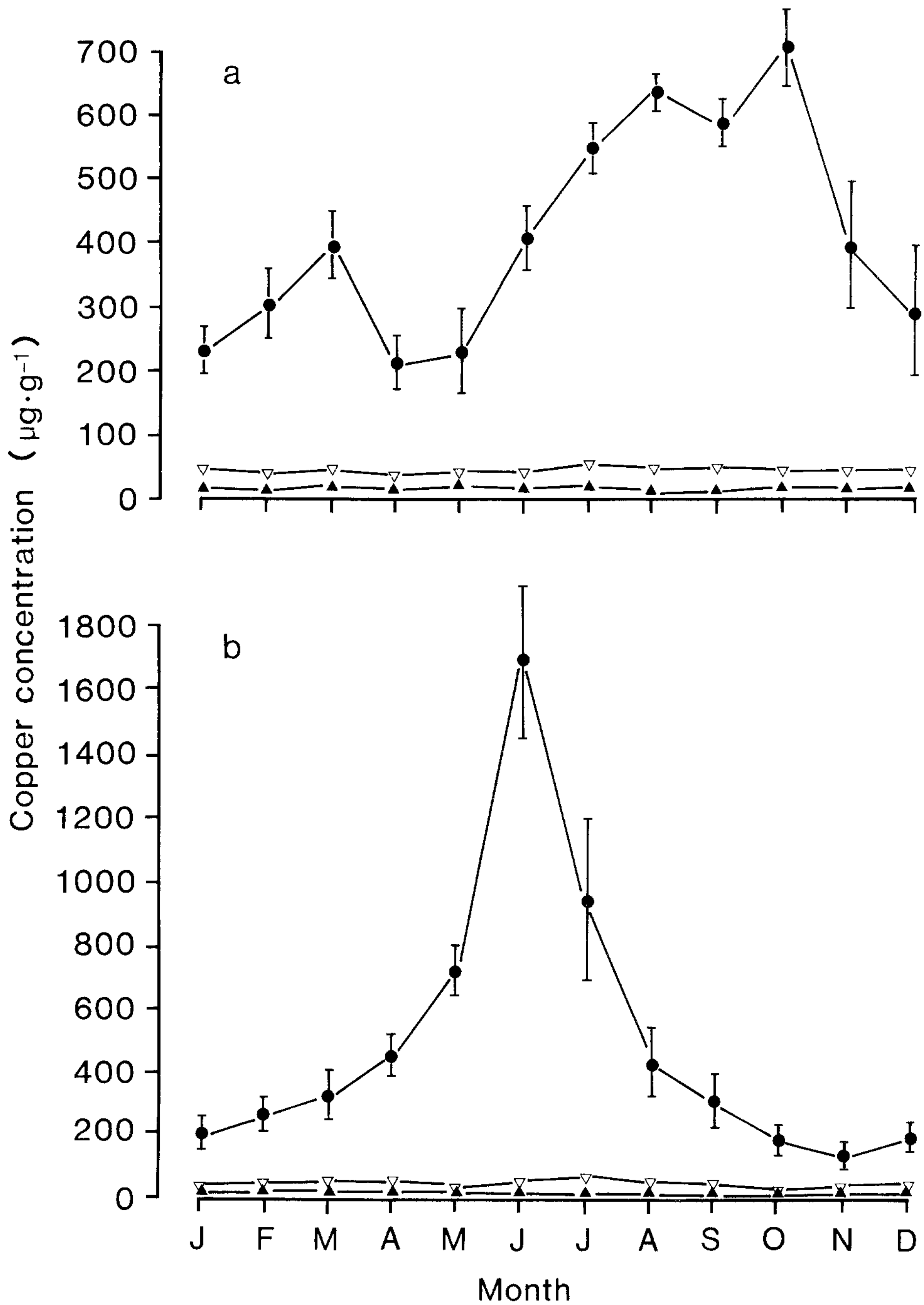


Fig. 10. Seasonal changes in Cu concentrations of (a) carabid and (b) staphylinid beetles at a refinery (●), 1 km (Δ) and control sites (■) (from Hunter *et al.*, 1987).

of Zn, but the concentration of the tolerant population was in balance with the food concentration of about 1373 ppm and the control population at a concentration of 392 ppm. The values were found to fall within the normal range found in the respective field situation, and the difference between the populations were due to a higher retention of Zn by the tolerant population. Thus, the body content of an individual also depends on the habitat it is raised in and the degree of tolerance in the population.

In general, Pb concentrations in soil invertebrates tend to be greater as contamination of their food increases. The element is mainly associated with calcified tissues and interacts with Ca in complex ways. Cadmium is extremely mobile and tends to concentrate in animals, especially in protein-rich tissues, where it may replace Zn in metalloproteins. Organism Zn tends to be higher at polluted sites, but the concentration ratio decreases as Zn in food increases, so that Zn concentration in soil animals varies less than Zn in their food between more or less contaminated sites.

It should be clear from this discussion that the concentrations of metals in soil animals cannot be accurately predicted from the concentrations in soil or litter, and that the concentration of metals in one species cannot be used to predict the concentration in another without proper knowledge about differences in diet, digestive system and storage/excretion mechanisms. Differences between species in susceptibility to metals in the field should at least partly be due to these differences in metal uptake, storage and turnover, and groups with a high storage/excretion capacity, such as isopods and collembolans, could be used to indicate availability of metals to soil animals in different environments. Such animals are, however, less affected by pollution than those with a low capacity, such as earthworms and carabids; the NOECs (No Observed Effect Concentration) are probably lower for the latter. As a consequence of the varying degree of immobility and availability of metals in the soil (in comparison with a water solution), 'critical' concentrations are more difficult to establish for soil invertebrates using the concentration in the soil as a reference than for freshwater or marine invertebrates, and a better approach would probably be to use the actual levels in body tissues as a reference.

4. Metal Effects on Life History Parameters; Growth, Mortality and Reproduction

The potential ecological impact of metals on soil invertebrates has often been estimated by field surveys. The usefulness of information on density, species number, diversity or distribution is often limited though, by the temporal and spatial patchiness of the soil fauna. Effects of metals on life history variables may be observed at low concentrations in laboratory experiments far ahead of effects on population density in the field (Bengtsson *et al.*, 1983a), so long-term effects on population densities could be predicted from data on the relation between metal concentration and population variables, such as the intrinsic rate of population growth (Bengtsson *et al.*, 1985a) and biomass turnover ratio (van Straalen and de Goede, 1987). On the other hand, the relevance of toxicological experiments in well defined laboratory cultures may be questionable (Bengtsson *et al.*, 1985a).

Decreased allometric growth is a common response in invertebrates in a metal polluted soil and has been observed for isopods (van Capelleveen, 1985, 1987a), snails (Russel *et al.*, 1981), collembolans (Bengtsson *et al.*, 1983a, 1985a), and earthworms (Bengtsson *et al.*, 1983b, Ma, 1983, 1984). van Capelleveen (1985) found that individuals of a local metal tolerant population of *Porcellio scaber* were about 20% smaller than those of a control population. This was at least partly due to a decreased consumption rate, since isopods ate less when their food was contaminated by more than 1900 mg kg^{-1} Zn or 390 mg kg^{-1} Zn and 10 mg kg^{-1} Cd (van Capelleveen 1987a). The same observations were made on the weight loss of the snail *Helix aspersa*, which was well correlated with its feeding rates (Figure 11), which were reduced by 100 ppm Cd or more (Figure 12) (Russel *et al.*, 1981).

Thus, the negative effect on growth may be explained by changes in food quality and availability. In a series of experiments with *O. armatus*, addition of food to a metal polluted soil was insufficient to compensate for decreased growth, unless the food was N enriched (Bengtsson *et al.*, 1985b). As moulting is a major metal excretion mehanism in Collembola and also closely connected with growth, extensive moulting may cause slower growth (Bengtsson, 1986). With a N enriched food source the costs of increased moulting frequency seemed to be compensated, and a high growth rate could be maintained even in a polluted environment. The moulting interval became short in *O. cincta* when fed Pb contaminated algae (Joosse and Verhoef, 1983) but not when the algae contained toxic levels of Cd (van Straalen *et al.*, 1989). Nonetheless, the allometric growth was the most sensitive parameter

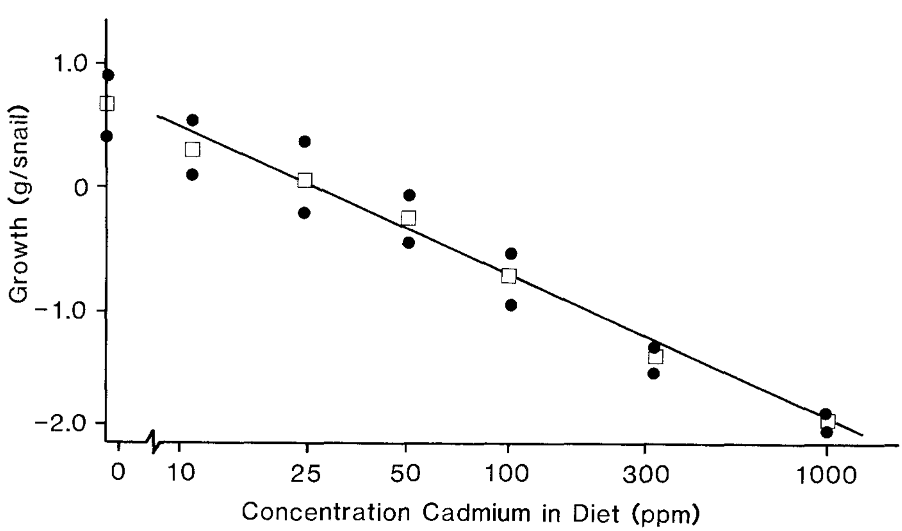


Fig. 11. Regression of growth of *Helix aspersa* versus ln [Cd]. Two lots of 25 snails (●) and mean (□) shown for each treatment (from Russel *et al.*, 1981).

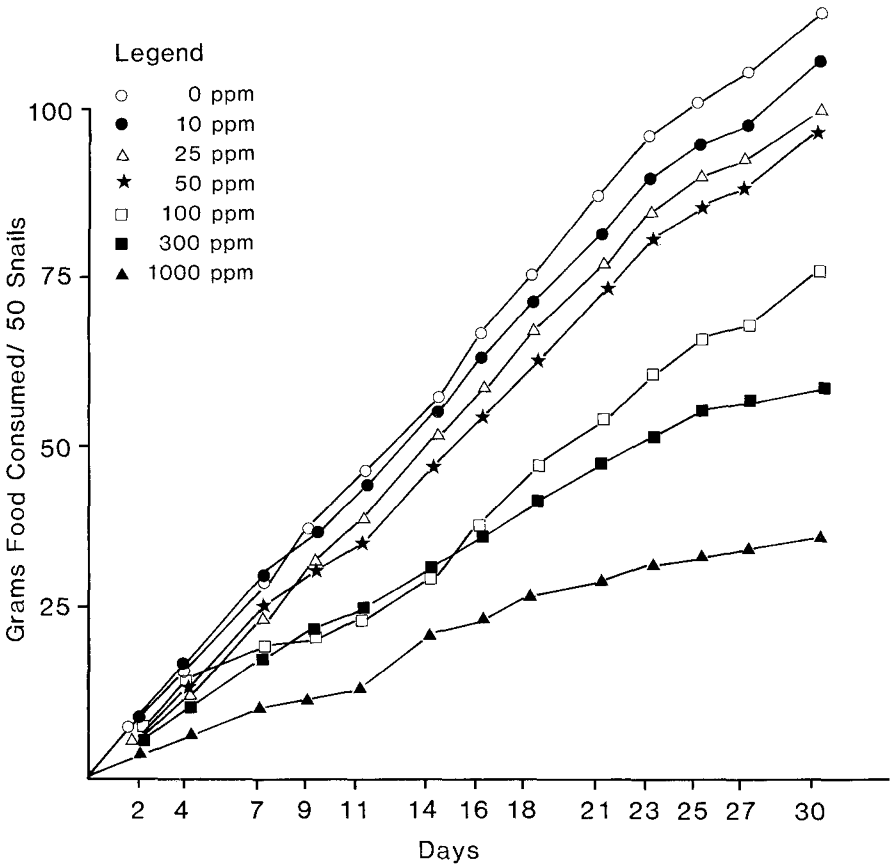


Fig. 12. Accumulated food consumption by Cd-treated and control *Helix aspersa* (from Russel *et al.*, 1981).

to Cd with negative effects expressed at 5 μg g^{-1}, and the authors speculated that other mechanisms, e.g. inhibition of growth hormones, may be responsible for the growth reduction. The oribatid mite *Platynothrus peltifer*, exposed to Cd in the same experiments, had the same NOEC value but the main effect was demonstrated on reproduction. Consequently, mites will more likely suffer from reduced population size in a Cd contaminated environment since individual reproduction is a prime driving variable for population growth. Groups with higher critical levels for reproductive effects, such as Collembola, should maintain population sizes longer in an environment that becomes Cd contaminated.

Earthworms suffered from a high juvenile mortality at the most polluted sites at Gusum (Bengtsson *et al.*, 1983b). Similarly, a slow volumetric growth and early juvenile mortality was observed in laboratory experiments. A growth test with newly hatched juveniles of *Lumbricus rubellus* showed that the rates of body growth and sexual development were progressively retarded with increasing levels of Cu in the soil (Ma, 1983). The critical concentration was about 60 ppm and a 50% reduction

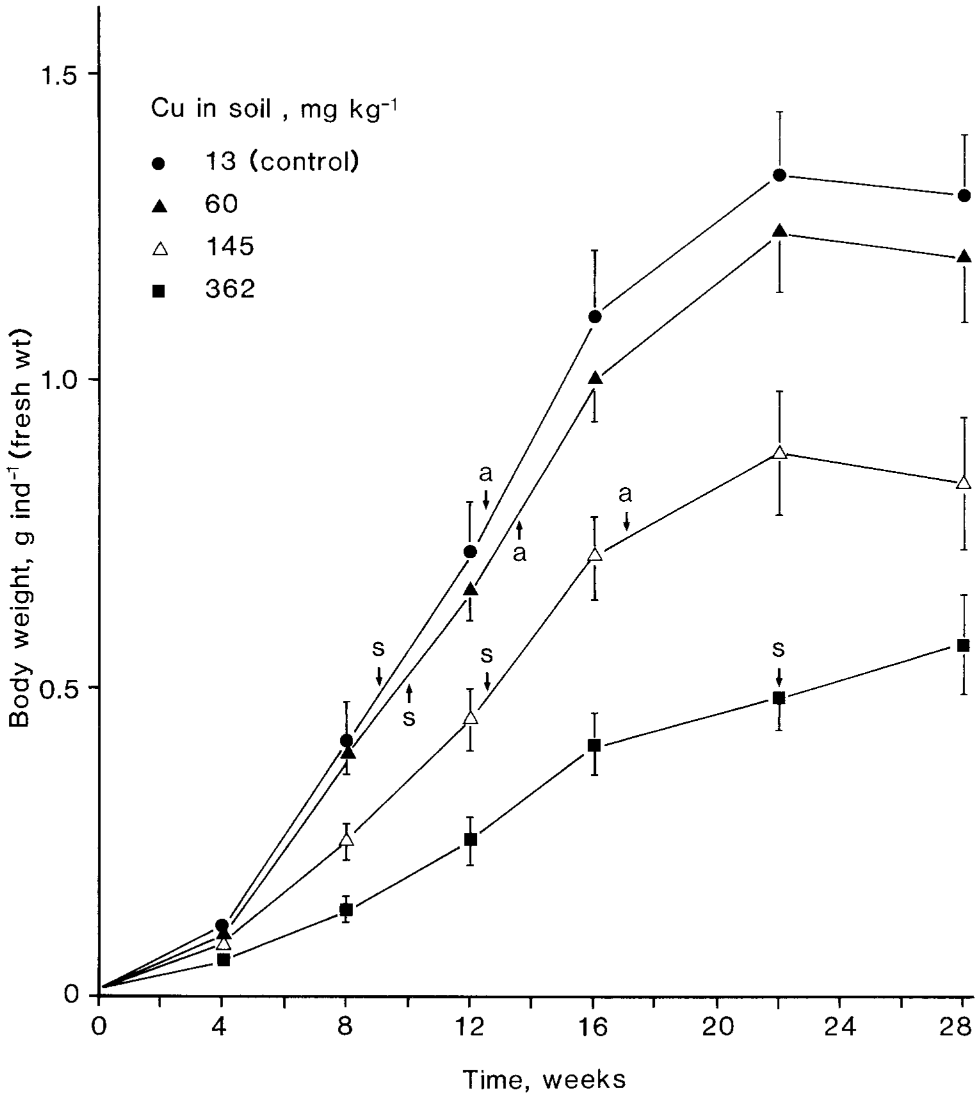


Fig. 13. Effect of Cu on growth and development of *Lumbricus rubellus* in a sandy loam soil. The arrows point to the time when 50% of the animals reached subadult (s) or adult (a) stage of development (from Ma, 1983).

occurred at about 150 ppm Cu (Figure 13). The same test with Cd yielded a critical concentration of about 20 ppm Cd, with a 50% reduction at about 60 ppm Cd in the soil. In a similar test with adult animals of the same species, body weight was the variable which was least affected (Table V) (Ma, 1984), weight gain was not reduced until the soil concentration of Cu was 300 $\mu g\ g^{-1}$.

Body size at first egg laying is relatively constant in Collembola (Bengtsson *et al.*, 1983a). Reduced growth and theoretical mean maximum length are thus of

TABLE V

Metal effects on life history parameters

Species	Lowest metal concentration with significant effect (mg kg^{-1})					Substrate	Parameter	Comment	Source
	Pb	Zn	Cu	Cd	Ni				
Eisenia foetida				50		horse manure	growth	$CdSO_4$	Malecki *et al.*, 1982
Eisenia foetida					200	horse manure	growth	$NiCl_2$	Malecki *et al.*, 1982
Eisenia foetida				100		horse manure	growth	$CuNO_3$	Malecki *et al.*, 1982
Eisenia foetida		2000				horse manure	growth	$ZnSO_4$,-NO_3,-Cl_2	Malecki *et al.*, 1982
Eisenia foetida	12000					horse manure	growth	PbAc	Malecki *et al.*, 1982
Eisenia foetida				25		horse manure	reproduction	CdAc	Malecki *et al.*, 1982
Eisenia foetida					200	horse manure	reproduction	$NiCl_2$	Malecki *et al.*, 1982
Eisenia foetida			100			horse manure	reproduction	$CuNO_3$-,SO_4	Malecki *et al.*, 1982
Eisenia foetida		500				horse manure	reproduction	$ZnSO_4$,-CO_3	Malecki *et al.*, 1982
Eisenia foetida	4000					horse manure	reproduction	$PbNO_3$,-Ac	Malecki *et al.*, 1982
Lumbricus rubellus adult				>300		sandy soil	mortality	$CuCl_2$	Ma, 1984
Lumbricus rubellus			60			sandy loam soil	no. of cocoons	$CuCl_2$	Ma, 1984
Lumbricus rubellus			130			sandy soil	litter breakdown	$CuCl_2$	Ma, 1984
Lumbricus rubellus			370			sandy soil	body weight gain	$CuCl_2$	Ma, 1984
L. rubellus (juv.)		60				sandy loam soil	growth	$CuCl_2$	Ma, 1983
L. rubellus (juv.)				20		sandy loam soil	growth	$CuCl_2$	Ma, 1983
Dendrobaena rubida		100				sand + cattle dung, pH 4.5	survival	$CuCl_2$	Bengtsson *et al.*, 1986
Dendrobaena rubida	100					sand + cattle dung, pH 4.5	survival	$CuCl_2$	Bengtsson *et al.*, 1986
Dendrobaena rubida			100			sand + cattle dung, pH 4.5–5.5	cocoon production	Pb-, Cd-, $CuNO_3$	Bengtsson *et al.*, 1986
Dendrobaena rubida				100		sand + cattle dung, pH 4.5–5.5	cocoon production	Pb-, Cd-, $CuNO_3$	Bengtsson *et al.*, 1986
Dendrobaena rubida	500					sand + cattle dung, pH 4.5–5.5	cocoon production	Pb-, Cd-, $CuNO_3$	Bengtsson *et al.*, 1986
Allolobophora caliginosa			110			? soil	cocoon production	?	van Rhee, 1975

Table V. (continued)

Species	Lowest metal concentration with significant effect (mg kg^{-1})					Substrate	Parameter	Comment	Source
	Pb	Zn	Cu	Cd	Ni				
Helix aspersa				100		food	growth	$CdCl_2$	Russel *et al.*, 1981
Helix aspersa				25		food	reproduction	$CdCl_2$	Russel *et al.*, 1981
Porcellio scaber		2000		10		food	brood success	Zn-, $CdNO_3$	van Capelleveen, 1987
Porcellio scaber		>400		>1		food	brood devlopm. time	Zn-, $CdNO_3$	van Capelleveen, 1987
Porcellio scaber		1000		>2		food	growth	Zn-, $CdNO_3$	van Capelleveen, 1987
Porcellio scaber	750	1800	50	50		O_2 litter	survival	approx. value	Beyer *et al.*, 1984
Porcellio scaber		3000				food	survival	ZnO	Joosse *et al.*, 1981
Porcellio scaber		1600				food	respiration	ZnO	Joosse *et al.*, 1981
Porcellio scaber		900				food	no. of progeny	ZnO	Joosse *et al.*, 1981
Porcellio scaber		900				food	juv. survival	ZnO	Joosse *et al.*, 1981
Porcellio scaber		1600				food	reproduction	ZnO	Beyer and Anderson, 1985
Porcellio scaber	12800					food	reproduction	PbO	Beyer and Anderson, 1985
Onychiurus armatus	3410			752		fungi	growth	Pb-, $CuNO_3$	Bengtsson *et al.*, 1983a
Onychiurus armatus	3089					fungi	survival	$PbNO_3$	Bengtsson *et al.*, 1985a
Onychiurus armatus			3245			fungi	survival	$CuNO_3$	Bengtsson *et al.*, 1985a
Onychiurus armatus	3410		752			fungi	survival	Pb-, $CuNO_3$	Bengtsson *et al.*, 1985a
Onychiurus armatus	1068					fungi	no. of eggs	$PbNO_3$	Bengtsson *et al.*, 1985a
Onychiurus armatus		3245				fungi	no. of eggs	$CuNO_3$	Bengtsson *et al.*, 1985a
Onychiurus armatus	2404	1512				fungi	no. of eggs	Pb-, $CuNO_3$	Bengtsson *et al.*, 1985a
Orchesella cincta				5		green algae	growth	$CdSO_4$	van Straalen *et al.*
Platyhothrus peltifer				3		green algae	reproduction	$CdSO_4$	1989

great importance for the reproductive success. In an experiment on metal effects on reproduction, animals compensated for the lower probability to survive by a smaller size at the reproductive start. van Capelleveen (1985) found a similar compensation in *P. scaber*, whose fecundity related to body-weight and size was higher in females from a polluted than a control site. In laboratory experiments a general negative and interactive effect of Zn and Cd was demonstrated on brood success, number of young released, and brood development time, of which the latter was the most sensitive parameter (van Capelleveen, 1985). Brood success declined at concentrations in the diet of >2000 mg kg^{-1} Zn and 10 mg kg^{-1} Cd, whereas brood development time was prolonged already between 400 to 2000 mg kg^{-1} Zn and 1 to 10 mg kg^{-1} Cd. Thus, observations of relatively fewer juveniles in contaminated soils in comparison with controls may not only be a consequence of enhanced juvenile mortality but also of decreased reproductive success of adults.

Van Rhee (1975) observed a lower cocoon production and egg viability in the earthworm *A. calliginosa* at 110 mg kg^{-1} Cu. In contrast, 100 mg kg^{-1} Cu was found to be beneficial for cocoon production in *D. rubida* (Bengtsson *et al.*, 1986). The explanation of this difference may partly be attributed to difference in response between the two species studied, but presumably mostly to differences in the amount of Cu available. In general though, an increased metal content reduced the number of cocoons deposited as well as the cocoon viability for *D. rubida.* It also changed the numbers of hatchlings per cocoon and increased the time for embryonic development. All the effects were magnified by reduced pH. Cocoon production in *L. rubellus* was also more sensitive to Cu than body weight or litter breakdown (Table V) (Ma, 1984).

Critical metal concentrations for life history variables, extracted from the literature, have been compiled in Table V. No one, so far, has systematically examined a great number of different factors influencing the susceptibility of a species, and data compiled should be evaluated with great caution. Our impression from comparing Tables IV and V is that data for combinations of different metals and soil conditions must have a profound impact on the criteria developed for critical loads. As it is rarely possible to demonstrate the effects of one single metal in field surveys, simulation experiments need to be performed in the field and in the laboratory to indicate the complex interactions between metals on the fate of individuals and populations. By combining and comparing the critical numbers in Tables IV and V we would suggest that the following environmental concentrations of metals should be used as a guide for further experiments on limiting levels, although we would prefer that the concentration in the animal tissue was used as a reference:

Pb	Cu	Zn	Cd	
100–200	<100	<500(?)	10–50	mg kg^{-1}

5. Tolerance and Adaptation

Changes in the environment exert a selective pressure on organisms to adapt to local conditions. Metal pollution can in that sense be compared with natural environmental changes, and genetical adaptation or physiological acclimatization may be expected in contaminated areas as metals exert a continuous selection pressure.

van Straalen *et al.* (1987) studied population differentiation in the collembolan *O. cincta* under the influence of metal soil pollution. Adaptation was measured as Pb and Cd excretion efficiency in individual Collembola from ten various sites.

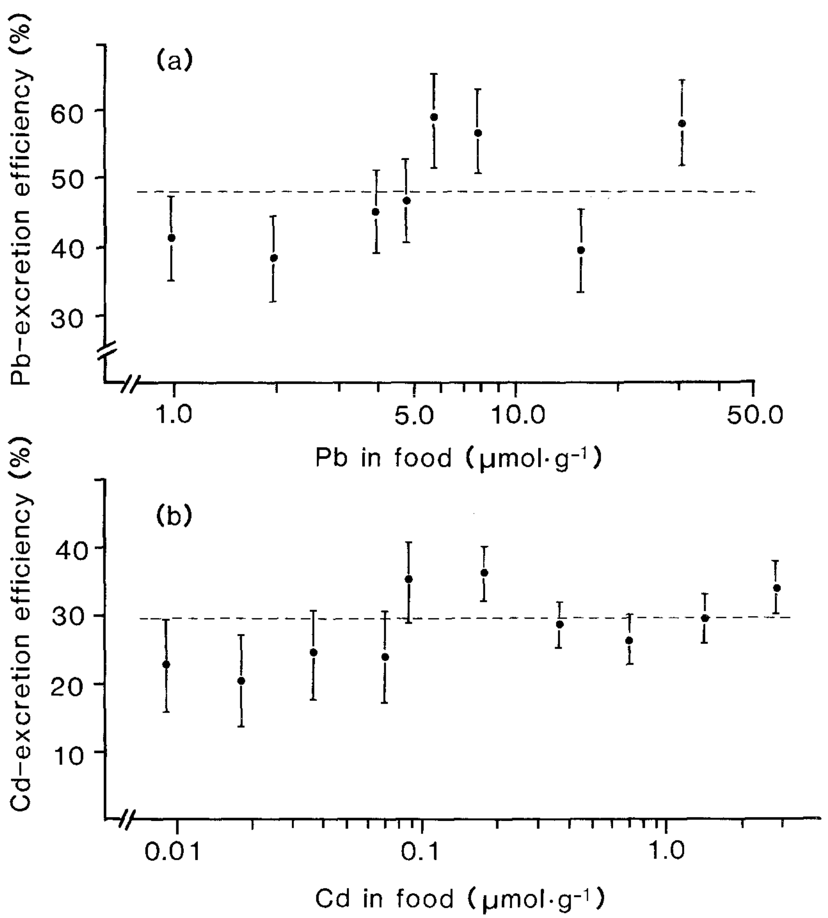


Fig. 14. Excretion efficiency of individual *Orchesella cincta* collected from various sites in the Netherlands. Populations are ordered from left to right according to the degree of contamination. (a) Pb excretion in three reference populations (R, D, H) and in three populations from Pb-contaminated areas (Z, P_1, S). (b) Cd-excretion in one reference population (R) and in five populations from Cd-contaminated areas (W, A, B, P_2, S). Each point gives the median for a population with a 95% confidence interval. Horizontal broken lines join populations that are not significantly different from each other (from van Straalen *et al.*, 1987).

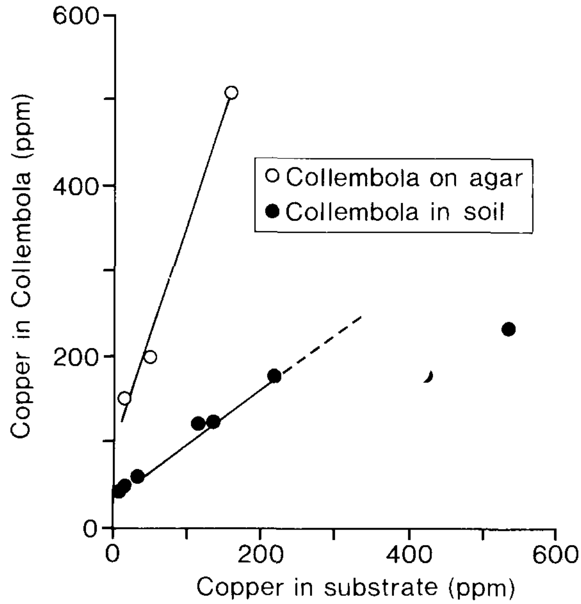


Fig. 15. Relations between concentration of copper in substrate (agar or soil) and concentrations in *Onychiurus armatus* (from Bengtsson and Rundgren, 1988).

Individuals inhabiting soils that had been contaminated for many centuries, such as the old mining site P in Figure 14, or to an extreme degree (the smelter site S in Figure 14) were able to increase their excretion efficiency, whereas moderate to high soil contamination of industrial origin initiated in the present century (site B and Z) decreased the excretion efficiency compared with that of reference sites (R, D, H). Most likely, the differences were not due to a physiological acclimatization, as the excretion was not influenced by experimental exposure to metals during several moulting intervals, but rather to long-term selection pressure inducing resistance. A similar indication of resistance was found in Gusum soils, where specimens of *O. armatus* at the most polluted sites had a lower Cu concentration than expected from the correlation between body and substrate concentrations for specimens in less polluted sites (Figure 15).

A population of the terrestrial woodlouse *P. scaber* collected from a Zn-polluted area was found to be adapted to high Zn and Cd concentrations (van Capelleveen and Joosse, 1987). Individuals from the polluted site produced larger quantities of metalloproteins and showed lower growth efficiences and drought resistance than individuals from a control site, and the 'tolerant' individuals were also on average 20% smaller. The energetic costs related to the tolerance were shown to be expressed as a trade off between tolerance and growth, so that tolerant individuals were less growth efficient in their habitat than control individuals in theirs – the tolerant individuals were, indeed, more growth efficient when they were fed Zn+Cd contaminated food than when fed uncontaminated food.

Differences in assimilation rates for Zn in two populations of the centipede *L. variegatus* were found to be related to the degree of contamination of the site from which the population was collected (Hopkin and Martin, 1984a). Centipedes from a contaminated site survived longer than those from an uncontaminated site when both populations were fed on woodlice hepatopancreas with high concentrations of metals. It is, however, not clear whether the differences between the populations were genetically based or whether the Zn regulation acclimated to the local metal levels during the life cycle of the animals.

Animals have evolved metal tolerance also by avoidance mechanisms (Joosse and Buker, 1979), and different studies have shown a capacity in soil invertebrates to discriminate between contaminated and uncontaminated food (Joosse *et al.*, 1981; Joosse and Verhoef, 1983; Tranvik and Eijsackers, 1989). The decreased consumption of polluted food may thus be an effect of an active avoidance mechanism which results in decreased growth if alternative good quality food is unavailable (van Capelleveen, 1987a; Russel *et al.*, 1981).

An adaptive response does not necessarily increase the chance of the individuals to survive but rather increases the probability of survival in an environment to which they are adapted (Moriarty, 1978). Thus, adaptation exerts a certain cost to the individuals, and the vulnerability of adapted populations to natural changes in the environment has been found to be enhanced (van Capelleveen and Joosse, 1987; Tranvik and Eijsackers, 1989). The physiological mechanisms for tolerance are not known, but in a variety of animals metal binding proteins have been found to be induced by metal pollution (Cooke *et al.*, 1979; Suzuki *et al.*, 1980; Morgan and Morris, 1982; Hopkin and Martin, 1984b; van Capelleveen and Faber, 1987) and are thought to be related to so called stress proteins which are synthesized in response to a variety of environmental stresses (Marx, 1983). The ability of populations to adapt to metals complicates the evaluation of field surveys in polluted areas as the activity or community characteristics will be less affected than may be expected from laboratory toxicity tests. This implies the importance of studies on adaptation in the field and of using the degree of tolerance as a tool in hazard assessment.

6. The Role of Animals in Decomposition Processes in a Metal Contaminated Soil

One of the first to recognize the disruption of forest decomposition processes by metals was Tyler (1972), but we know of only four works that specifically address the relationships between soil invertebrates and decomposition in metal contaminated substrate. Jackson and Watson (1977) found that litter accumulation in the fermentation layer (O_2) could be measured at a lower degree of contamination (6800 mg Pb, 20 mg Cd, 351 mg Zn, 113 mg Cu kg^{-1} litter) in a transect from a lead smelter in Missouri than the reduction of the biomass of litter arthropods in general (at 30 500 mg Pb, 60 mg Cd, 917 mg Zn, 448 mg Cu kg^{-1} litter). The pool of Ca, Mg, K and P in litter and soil was depleted at the same metal concentration

TABLE VI

Relationships between metal concentrations, arthropod abundances, and litter decomposition. From Strojan, 1978b

Distance from smelter (km)	1	6	40	
Metals in litter (mg kg^{-1})				
Zn	25750	14600	676	
Cd	885	256	88	
Cu	340	172	47	
Pb	2333	971	258	
total arthropods extracted (%)		18.1	55.1	100
oak leaf weight loss (%)	19.1	25.7	36.8	
litter layer thickness (cm)		12.4	7.0	6.0

TABLE VII

Relationships between Cu concentration and weight loss of alder leaves due to the activities of the earthworm *L. rubellus*. From Ma, 1984

Cu (mg kg^{-1})	13-14	54-63	131-136	372-373
pH 4.8	18.4	16.9	14.8	7.3
weight loss				
pH 7.3	10.5	9.2	8.1	6,8

as the arthropods became fewer. A closer agreement between litter accumulation and decomposer density and activity would probably be attained by an extended resolution of their biological data.

Strojan (1978b) found a positive correlation between the numbers and diversity of soil microarthropods and the first-year weight loss of litter in litter bags placed at three sites along a transect from the zinc smelter at Palmerton (Table VI). The decline in the number of mites near the smelter was particularly striking. The litter bag method probably underestimated the litter loss *per se*, since the 1.5 mm mesh size bags excluded larger soil invertebrates, such as isopods, earthworms and beetles. As a consequence, the impact of metals on the decomposition might also be underestimated, since also the macrofauna would be reduced near the smelter. As the litter bag experiment did not include any manipulation to separate the contribution by soil microarthropods and microorganisms to litter weight loss, the effects observed were most likely due to a reduction in the numbers and activity of both animals and microorganisms.

In the experiments by Ma (1984) on the sublethal toxicity of Cu to the earthworm species *L. rubellus*, the weight loss of alder leaves was significantly reduced at 130 mg kg^{-1} Cu added as copper sulphate after 6 weeks of incubation in a sandy soil at pH 4.8 and in a sandy loam at pH 7.3 (Table VII). As a comparison cocoon production by the worms was slightly more sensitive as a test variable; it was inhibited at 130 mg kg^{-1} at pH 4.8 and at 65 mg kg^{-1} at pH 7.3. This effect of pH on Cu toxicity was the opposite to what is commonly found and may be related to the low activity shown by *L. rubellus* in loam soil compared with sandy soil.

Bengtsson *et al.* (1988) were able to differentiate the contribution of enchytraeids and microarthropods to mass lost and mineralization in soil columns from two sites in the Gusum area incubated for 10 weeks under controlled laboratory conditions. Animals present in the control soil columns (7.8 km from the mill; cf. Table III) increased C and N mineralization and enhanced leaching of dissolved organic C and nutrients by 20 to 30% over that attributed to microbial effects. The overall decomposition rate decreased by 20% in the columns with a Zn+Cu concentration that was approximately 100 times above the background (275 m from the mill; cf. Table III); all of the reduced mass lost and 20 to 35% of the reduction of leached organic C and inorganic nutrients could be explained by the reduced animal activity. It was predicted that it would take 100 yr to remove 50% of the organic matter in the litter in the polluted compared with 6 to 7 yr in the unpolluted soil, and the authors estimated that their soil column method, with five replicates, would have a 18% difference in mass lost as a detection limit. That would correspond to a Zn+Cu concentration of about 500 mg kg^{-1}, twice the background at Gusum. Twenty replicates or more would be required to discover a mass loss due to a 50% increase of the metal concentration.

Although the microcosm method may have the capability to reveal effects on decomposition by as little as a twofold increase of metal concentrations, the quantitative relationships between metal concentration and availability, soil animal abundance and activity, and decomposition rates are very vague and should be seriously addressed in future research along with studies on the consequences of reduced decomposition on root activities. As long as the knowledge of mechanisms of interactions between soil microorganisms and invertebrates, mechanisms of action of metals on the soil biota and mathematical descriptions of the decomposition rates is premature, and until multiple-year field studies have been conducted, the forecasting of the fate of metal deposition on soil decomposition will remain uncertain.

7. General Conclusions

Very few, if any, of the surveys and experiments presented in this review were originally designed for quantitative assessment of critical loads in the environment. Most of them were merely undertaken to demonstrate unequivocal effects of metals on soil invertebrates, and in most cases they were quite successful. Metals seem to have profound effects on biological variables at any organization level, whether cells, tissues, organisms, populations or 'communities', but with respect to critical loads the effects are essentially qualitative. The NOEC values we suggest are probably conservative and without reference to long-term consequences on e.g. decomposition and nutrient regeneration. On the other hand, the process of induced selection of metal tolerance is completely ignored.

The data base is generally very weak, and one would wish that at least some systematically quantitative work had been done, neatly joining carefully replicated field site descriptions and manipulative experiments to elucidate processes and mechanisms affected by metals. Sometimes in the future it may be possible to connect quantitative effects on life history parameters with the structure and function of the species assemblages to forecast the fate of populations and long-term productivity, and to back it up with some few more models and observations of decomposition changes and induced local adaptations to changed metal concentrations.

Meanwhile, some guidelines could be developed to facilitate the use of field and laboratory data in hazard assessment. The assessment would approach scientific rigour once the supporting data become more quantitative and detailed. It would help if the guidelines specified what variables that would be required for more precise predictions of the effects of metals on soil invertebrates and their activities.

As a first approximation the NOEC value for a specific metal could be normalized for the relative toxicities of metals to soil biota in general, which we can express as NOEC (μmol g^{-1}) = [Pb] + 6[Cd] + 5[Zn] + 5[Cu], where the multiplication factors originate from a discussion with Doelman and Hopkins, among others. As an example, NOEC of only Zn for earthworms observed by Bengtsson *et al.* (1983b) in soils contaminated by 170 mg Zn, 78 mg Cu and 36 mg Pb kg^{-1} can be calculated as 253 mg kg^{-1}. The earthworms were collected in a soil of pH 5.5, and we have to divide the NOEC value obtained by a species specific function of pH to express the pH defined NOEC. From Bengtsson *et al.* (1983b) we can estimate that performance of the earthworms at pH 5.5 and 4.5 is 80% and 30% of their performance at pH 6.5. Consequently, NOEC ($Zn_{pH4.5}$) will be $3/8 \times 253 = 95$. In another soil with pH 4.5 and 150 mg kg^{-1} Cu in addition to Zn we would have to set a lower limit, *viz.* 31 mg kg^{-1}, where we have also normalized for the pH related Cu effect on the earthworms (a factor 2/8).

Other factors that influence the NOEC values are cation exchange capacity, organic matter and clay content. For the latter two the Dutch Ministry of Housing, Physical Planning and Environment has suggested normalization factors for NOECs for a standard soil, e.g. 0.4 + 0.007 [clay (%) + 3xOM (%)] for a reference value on Cd (cited in van Straalen and Denneman (in press)). van Straalen and Denneman used the corrections to calculate NOEC values of Cd in standard soil (25% clay and 10% organic matter) for reproduction of various soil animals; for *D. rubida* (Bengtsson *et al.*, 1986) 154 μg, *E. foetida* (Malecki *et al.*, 1982) 13.8 μg and for *P. scaber* (van Capelleveen, 1987b) 3.3 $\mu g\ g^{-1}$ (cf. Table V). The authors also suggested a model that would allow risk assessment by NOEC values that vary from one species to another, so that a certain protection level, e.g. covering 95% of all species, can be choosen.

A final step in developing critical levels for soil animals would be to incorporate a frequency distribution of the number of observations around critical levels in a probability distribution to express the confidence in accepting a specific value.

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