Ecological Bulletins 39: 53–56. Copenhagen 1988

Toxicity of copper to lumbricid earthworms in sandy agricultural soils amended with Cu-enriched organic waste materials

Wei-chun Ma, Research Institute for Nature Management, P.O. Box 9201, 6800 HB Arnhem, The Netherlands

The average copper (Cu) content of Dutch arable sandy soils is 11 mg kg^{-1} (van Driel and Smilde 1981). Normal background concentrations of Cu may be increased appreciably by anthropogenic activities, for example in urban or industrial areas or in agriculture.

The use of copper sulphate as a feed additive to improve the efficiency of pig production has led in recent years to the spreading of Cu-enriched pig slurry on land. This has become a major emission source of copper in The Netherlands, with local inputs of between 1 and 10 kg Cu $ha^{-1} yr^{-1}$ in areas with intensive pig production. Furthermore, high local Cu inputs are generated in many countries by the spraying of copper oxychloride as a fungicide in fruit-growing areas. Other important pollution sources of Cu include dredged sludges, sewage sludges, and composted municipal wastes, which are often applied to sandy agricultural soils as organic fertilizer. Sewage sludge, for example, may contain Cu in concentrations between 200 and 8000 mg kg^{-1} dry matter (Berrow and Webber 1972). In The Netherlands, sewage sludge containing Cu at levels of up to 600 mg kg^{-1} dry matter is permitted for use in agriculture at annual rates of 1 and 2 tonnes dry matter per ha on grassland and arable soil, respectively. Similar guidelines are used for the application of composted municipal wastes.

Added Cu accumulates in the top 0–25 cm layer of arable land and in the top 0–5 cm layer of permanent grassland. In the long run, a level may be reached at which irreversible detrimental effects on plant and animal life occur. Since this accumulation of Cu can affect the ecological functioning of the soil and reduce soil fertility, it has become necessary to establish soil quality standards with respect to environmentally safe levels of copper. For this purpose, we need ecotoxicological data as a basis for environmental impact assessment leading to the regulation of copper use in agriculture and organic waste management.

Field observations (Niklas and Kennel 1978, van Rhee 1975) have suggested that copper is detrimental to lumbricid earthworms. This assumption is supported by laboratory studies, which showed that the toxicity of copper to earthworms is influenced by the pH and organic matter content of the soil (Streit and Jaeggy 1983, Streit 1984, Ma 1984). One of the objectives of the study reported here was to establish the specific contribution of copper to the toxicity to earthworms of organic waste materials such as sewage sludge, pig slurry, and composted urban wastes applied to sandy soils. In addition, the effect of pH was investigated further and a coherent analysis of various laboratory and field data was made to determine acceptable levels of soil Cu in relation to toxicity to earthworms.

Materials and methods

Laboratory and field experiments were carried out in sandy agricultural soils with pH 4.8–5.2, clay 2–4%, organic matter 4–6% and CEC 5–9 meq. (100 g dry soil)$^{-1}$. For a field experiment performed on arable land, composted urban waste was mixed into the 0–20 cm plough zone once a year at various rates. Sulphur or calcium carbonate was used to adjust the pH of the soil. Samples were taken after a lag period of three to four years during which no waste compost had been added. Sampling for earthworms was done in two to four replicate plots by digging and hand-sorting followed by additional formalin extraction.

Pot experiments were performed in four to six replicates at 15°C in 5-litre containers each provided with 6 kg (dry weight) soil and five worms. The median effective concentration at which a test parameter (e.g., reproduction) was reduced by 10% (EC_{10}) or 50% (EC_{50}) relative to the untreated control was determined by fitting generalized logistic response curves according to a computerized least-squares method. Behaviour experiments were conducted in 2-litre glass containers, one half of each was filled with Cu-amended and the other half with unamended soil. Five worms were placed on the surface of the soil in each jar, and the worms and all burrows made in both compartments were photographed at 24-h intervals to record the position of the worms and the length of the tunnels. Total burrow

9466

Tab. 1. Effect of copper on earthworm cocoon production determined after 4 weeks of exposure to sandy soil with added $CuCl_2$ at 15°C.

Test species	Cu concentration in soil (mg kg^{-1})			
	EC_{10}	95% confidence interval	EC_{50}	95% confidence interval
Lumbricus rubellus	80	44–147	122	94–157
Aporrectodea caliginosa	27	17–45	68	55–83
Allolobophora chlorotica	28	21–38	51	46–57

Tab. 2. Distribution (in %) of total burrow length and frequency of occurrence of *Lumbricus rubellus* given a choice between a compartment containing amended ($CuSO_4$) soil and a compartment with unamended soil.

Test parameter	Soil	Cu conc. in amended soil (mg kg^{-1})			
		50	100	200	300
Burrow length	Unamended	56	59	75	92
	Amended	44	41	25	8
Worm distribution	Unamended	64	75	81	96
	Amended	36	25	19	4

length reached a peak level after about two days. Average burrow length and worm distribution were calculated for days 3 and 4. The experiment was done in four replicates.

The metal content of soil and worm samples in all laboratory and field experiments was determined by atomic absorption spectrophotometry after digestion with H_2SO_4-HNO_3 (1:1); concentrations are expressed on a dry matter basis.

Results and discussion

Laboratory studies

Tab. 1 shows the results of pot experiments performed to determine the effect of copper alone on reproduction rate of three species of earthworms. In *Aporrectodea caliginosa* and *Allolobophora chlorotica* cocoon production started to decline at a soil Cu level of about 30 mg kg^{-1} and in *Lumbricus rubellus* at 80 mg kg^{-1}. EC_{50} values ranged from about 60 mg kg^{-1} for *Aporrectodea caliginosa* and *Allolobophora chlorotica* to about 120 mg kg^{-1} for *L. rubellus*. The former two species thus appeared to be somewhat more sensitive to Cu. Furthermore, they showed an increased tendency to become quiescent when toxic environmental conditions were encountered.

Soil pH had a significant influence on the toxicity of Cu for lumbricids. Fig. 1 shows the effect of pH on the toxicity of Cu in two species, *Aporrectodea caliginosa* and *Lumbricus rubellus*. *A. caliginosa* seems to be a more acid-sensitive species than *L. rubellus*. The difference in acid sensitivity can be seen from the specific effect of pH on reproduction in unamended soil (12 mg Cu kg^{-1}), where pH-dependent reproduction was seen in *A. caliginosa* but not in *L. rubellus*.

In Cu-polluted soil, however, both species showed a pH response. This is due to the physico-chemical environment, which determines the chemical form of the

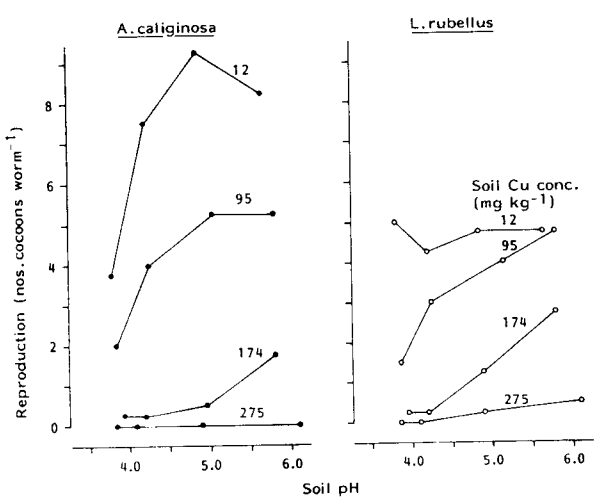


Fig. 1. Influence of pH on the effect of Cu in soil on the reproduction of two earthworm species.

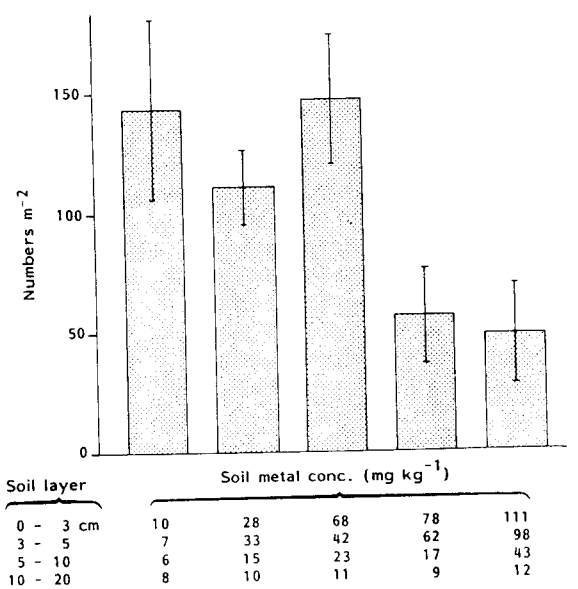
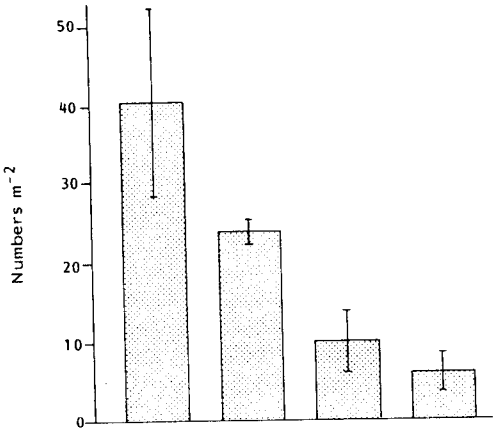


Fig. 2. Effect of frequent spreading of pig slurry on grassland on the distribution of Cu in the soil profile (lower part of Fig.), and the population size of *Lumbricus rubellus* (upper part of Fig.).



	Soil layer	Soil metal conc. (mg kg^{-1})			
Cd	0 - 3 cm	0.7	15	31	57
	3 - 5	0.5	10	21	31
	5 - 15	0.5	2.6	5.1	5.7
	15 - 25	0.3	0.5	0.7	0.8
Cu	0 - 3 cm	5.5	69	138	265
	3 - 5	4.7	50	99	150
	5 - 15	3.9	13	23	25
	15 - 25	3.0	4.5	5.4	6.0
Pb	0 - 3 cm	23	79	144	254
	3 - 5	22	147	288	438
	5 - 15	19	49	79	89
	15 - 25	16	21	22	24
Ni	0 - 3 cm	3.6	30	57	101
	3 - 5	4.7	24	44	69
	5 - 15	3.9	8.6	15	17
	15 - 25	2.7	4.1	3.5	3.9

Fig. 3. Effect of frequent spreading of sewage sludge on grassland on the distribution of some important heavy metals in the soil profile (lower part of Fig.) and the population size of *Lumbricus rubellus* (upper part of Fig.).

copper in the soil and hence the toxicity of the metal to organisms. Measurements of Cu at various pH levels in the soil show that the activity of cupric ions decreased with increasing pH, independent of the concentration of Cu present.

An important question to investigate was whether earthworms would avoid migrating into sites polluted with Cu. In behaviour experiments a choice was given between untreated and treated soil. The results, which are summarized in Tab. 2, indicate that lumbricids are capable of distinguishing between Cu-polluted and unpolluted soil. The average burrow length made in polluted soil decreased with increasing Cu concentration, and the less frequent presence of worms reflected their reluctance to enter the polluted soil. This avoidance behaviour may have implications in the field, because it means that there is less chance of recolonization of Cu-polluted sites. It also suggests that caution should be exercised before concluding what chance earthworms have of surviving in Cu-polluted soil when the homogeneity of the pollution in the soil is uncertain and a refuge into unpolluted micro-sites remains possible.

Field studies

Figs 2 and 3 show the results of field experiments carried out in grassland receiving sewage sludge and Cu-enriched pig slurry, respectively. In both cases the sampled population showed *L. rubellus* as the dominant species. The data in these figures show that Cu accumulates strongly in the upper soil surface horizons. In the case of sludge-amended soil other metals accumulate as well; data on some of these metals are included in Fig. 3. The decline of the lumbricid populations was found to be correlated with an increase in the concentration of metals retained in the uppermost soil layer (0–5 cm). In both experiments a lag period of three to four years was allowed during which no treatment was applied before sampling took place. The outcome reflects the long-term consequences of soil treatment with sewage sludge or pig slurry.

Fig. 4 shows the results of a field experiment in arable soil with added urban waste compost and various pH levels. Sampling was done after an untreated lag period of four years. The population was composed predominantly of *Aporrectodea caliginosa*. The upper 0–20 cm layer of the soil showed an accumulation of Cu together with a number of other metals. The population decreased in numbers with increasing concentrations of metals in the soil. However, the toxic effect was substantially alleviated by liming of the soil. The influence of pH is presumably related to the modifying effect of pH on the available form of metals in metal-polluted soil (Emmerich et al. 1982). This influence of pH is similar to the effect of pH on Cu toxicity described

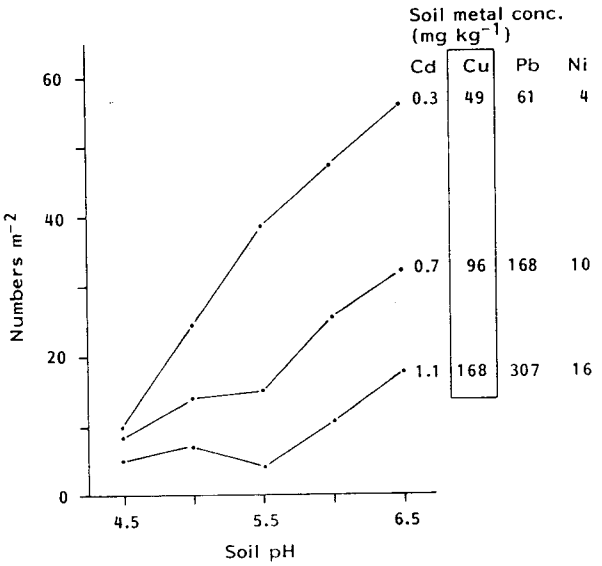


Fig. 4. Influence of pH on the effect of composted urban waste incorporated into the ploughed layer (0–20 cm) of arable soil on the populations size of *Aporrectodea caliginosa*. The accumulation of some heavy metals in the 0–20 cm layer is also shown.

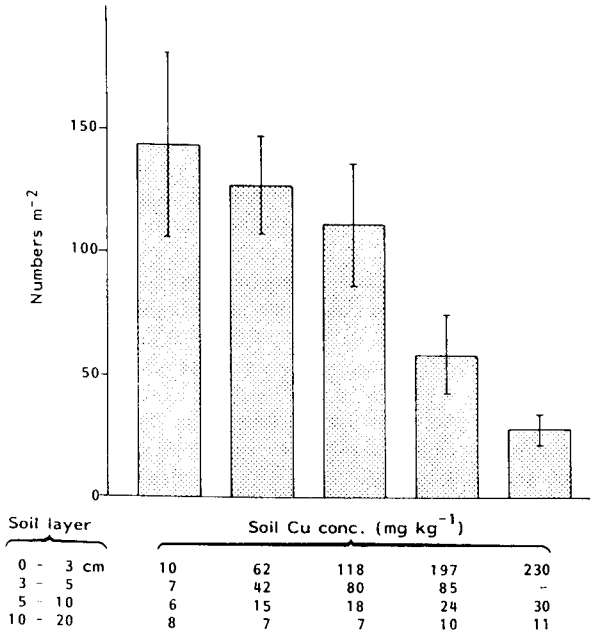


Fig. 5. Effect of frequent spreading of copper sulphate on grassland on the distribution of Cu in the soil profile (lower part of Fig.) and the population size of *L. rubellus* (upper part of Fig.).

above for reproduction of *Aporrectodea caliginosa* in the laboratory (see Fig. 1).

In plots treated with pig slurry, sewage sludge, or composted urban wastes the specific effect of Cu is difficult to assess because of the simultaneous presence of other metals. Sewage sludge and composted urban wastes contain many different kinds of heavy metals, and in pig slurry the salt concentration or the release of ammonia or other toxic substances may exert a harmful effect in addition to that of Cu. Nevertheless, the toxic effect of Cu alone could be assessed indirectly by comparing dose-response relationships obtained for sewage sludge or urban compost with those obtained in field experiments with copper as the only contamination source.

Fig. 5 shows the decrease in the size of a population of predominantly *L. rubellus* in grassland treated annually with only copper sulphate at rates comparable to those in the field experiments on Cu occurring in sewage sludge and pig slurry. The relative population decline associated with exposure to copper sulphate occurred at higher concentrations of Cu in the soil surface horizons than would have been expected from the results shown in Figs. 2 and 3 for Cu in sewage sludge or pig slurry. This suggested that although Cu was the main factor underlying the population decline, pig slurry and sewage sludge have other compounds that exert some additional toxic effect.

One of the consequences of frequent applications of Cu-enriched pig slurry is the formation of a mat of plant debris in the soil surface horizon (McGrath et al. 1980). The present results can explain this phenomenon, i.e., a strong reduction of populations of lumbricids by Cu leads to a diminished rate of breakdown of dead plant litter, which would then accumulate on the soil surface.

Concluding remarks

The present study has shown that the accumulation of Cu in the soil affects the reproductive potential of earthworms and at the same time deters earthworms from recolonizing Cu-polluted soils. The combined effect of these two responses would be a strong and sustained decline of lumbricid populations at Cu-polluted sites. Comparison of soils contaminated with single metal or multiple metals showed that Cu accounts for the greater part of the population decline occurring in soils treated with sewage sludge or composted urban wastes.

It was also found that in unlimed sandy agricultural soils (with about 4 to 6% organic matter and a pH of about 5) detrimental effects on earthworm populations can be expected to start at Cu concentrations above 50 mg kg^{-1} with a drastic decline above 100 mg kg^{-1}. Treatments to rehabilitate Cu-polluted soils with respect to toxicity for earthworms might benefit from the inclusion of liming to raise the pH.

References

Archer, F. C. 1980. – Proc. ADAS Conf. on Inorganic Pollution and Agriculture, HMSO, London.

Berrow, M. L. and Webber, J. 1972. Trace elements in sewage sludges. – J. Sci. Fd. Agric. 23: 93–100.

Driel, W. van and Smilde, K. W. 1981. Heavy-metal contents of Dutch arable soils. – Landwirtsch. Forsch., Sonderheft 38: 305–313.

Emmerich, W. E., Lund, L. J. and Chang, A. C. 1982. Predicted solution phase forms of heavy metals in sewage sludge-treated soils. – J. Environ. Qual. 11: 182–186.

Ma, Wei-chun 1984. Sublethal toxic effects of copper on growth, reproduction and litter breakdown activity in the earthworm *Lumbricus rubellus*, with observations on the influence of temperature and soil pH. – Environ. Pollut. (A) 33: 207–219.

McGrath, D., Poole, D. B. R. and Fleming, G. A. 1980. Hazards arising from application to grassland of copper rich pig faecal slurry. – In: Gasser, J. K. R. (ed.) Effluents from livestock. Applied Science Publishers Ltd., London, pp. 420–431.

Niklas, J. and Kennel, W. 1978. Lumbricidenpopulationen in Obstanlagen der Bundesrepublik Deutschland und ihre Beeinflussung durch Fungizide auf Basis von Kupferverbindungen und Benzimidazolderivaten. – Z. PflKr. PflSchutz 85: 707–713.

Rhee, J. A. van 1975. Copper contamination effects on the earthworms by disposal of pig waste in pastures. – In: Vaněk, J. (ed.) Progress in Soil Zoology. Proc. 5th Int. Soil Zool. Colloq., Prague: 451–457.

Streit, B. 1984. Effects of high copper concentrations on soil invertebrates (earthworms and oribatid mites). – Oecologia (Berl.) 64: 381–388.

– and Jaeggy, A. 1983. Effect of soil type on copper toxicity and copper uptake in *Octolasium cyaneum* (Lumbricidae). – In: Lebrun et al. (eds). New trends in soil biology, Louvin-la-Neuve, pp. 569–575.