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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

Wei-chun Ma

Research Institute for Nature Management,
Kemperbergerweg 67, 6816 RM Arnhem, The Netherlands

ABSTRACT

The sublethal effects of copper on Lumbricus rubellus *Hoffm. have been investigated with respect to growth, cocoon production and litter breakdown activity. The concentrations of Cu in the soil ranged from 13 to 370 mg kg^{-1}. Copper additions to soil caused the greatest decreasing effect on cocoon production. Significant decreases in cocoon production at 2 to 6 weeks (15 °C) were found at concentrations in the range of 100 to 150 mg Cu kg^{-1}, applied either to a sandy soil or to a sandy loam. Litter breakdown activity responded less sensitively with significant decreases occurring at 150 to 300 mg Cu kg^{-1}. Body weight was least affected; significant reduction of body weight gain occurred at concentrations greater than 300 mg Cu kg^{-1}, together with the first occurrence of mortality. The copper toxicity observed in the acid sandy soil decreased as the soil pH was adjusted from 4·8 to 7·1. Cocoon production increased and body growth decreased as the temperature increased from 12 to 18 °C. Litter breakdown activity at 6 weeks showed an optimum at 15 °C. The temperature response remained largely unaltered in Cu-amended soil. The relative effect of copper was generally greater as the temperature decreased. These studies show that copper may seriously affect earthworm populations. They also demonstrate the potential usefulness of* L. rubellus *as a test species in ecotoxicological evaluation of environmental pollutants in soil.*

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INTRODUCTION

Major man-made sources of soil pollution with copper include the application to land of sewage sludge and other municipal and industrial wastes and the agricultural use of copper-containing fungicides. As reviewed by Tiller & Merry (1981), the widespread use of copper as a feed additive to improve the efficiency of pig production can also considerably increase Cu concentrations in soil when copper-contaminated pig slurries are continually spread on land.

Copper has often been studied with respect to phytotoxicity and crop production, but little is known about the detrimental effects on animal life in soil. The objective of the present study was to investigate the toxicity to earthworms as an ecological indicator of the environmental impact of soil pollution, involving an important component of the soil invertebrate fauna. The rôle of earthworms in organic matter breakdown and incorporation of organic matter into the soil, as well as the contribution of earthworms to soil structural improvement, are widely recognised (Edwards & Lofty, 1977).

Available data on copper toxicity to earthworms are scarce and the evidence obtained has been mostly of an indirect nature. Nielson (1951) observed from an accidental spill of copper sulphate in grassland that earthworm populations were reduced by half at a concentration of 150 mg Cu kg^{-1} in the surface soil, with an almost total elimination at 260 mg Cu kg^{-1}. Van Rhee (1967) reported a gradual decline of earthworm populations in orchard soil with 85 mg Cu kg^{-1} accumulated in the upper soil layer as a consequence of past spraying with copper-containing fungicides. Reductions of earthworm populations in orchard soils have also been linked to the application of copper-containing fungicides in several other studies (Raw, 1959; Hirst *et al.*, 1961; Niklas & Kennel, 1978). Preliminary laboratory observations have indicated that the 6-week LC_{50} of copper, applied as copper chloride to sandy loam soil, is at about 1000 mg Cu kg^{-1} for adult worms of *Lumbricus rubellus* (Ma, 1982). Sublethal effects have been described in the compost worm, *Eisenia foetida*, by Malecki *et al.* (1982), who found that copper added as copper chloride to animal manure inhibited growth and cocoon production at concentrations of 500 to 2000 mg Cu kg^{-1}.

The present study describes sublethal toxicity effects of copper on the soil-inhabiting earthworm, *L. rubellus*, both in terms of growth, reproduction and litter breakdown activity. Additional observations are reported on the influence of temperature and soil pH.

MATERIALS AND METHODS

Adult worms of *Lumbricus rubellus* Hoffm. were collected from a continuous grassland site. Experimental units were formed consisting of nylon-meshed netbags with a 5-litre volume of soil, each unit containing five worms of about equal size and weight. To ensure adequate space, the height of the soil column in each bag was kept at 20 cm. A measured amount of air-dried alder leaves was placed on top of the soil surface in each unit. Unless otherwise stated, experiments were done in an agricultural sandy soil (loamy sand) consisting of 2% clay, 20% silt and 5·7% organic matter; pH (KCl) was 4·8 and soil moisture content, 15%. The units are embedded into a layer of soil in large containers and kept at a constant temperature of 15 °C and 90% air humidity. Observations were made on: (1) mortality rates; (2) cocoon production per unit, as determined by wet-sieving and hand-sorting; (3) litter breakdown activity per unit, as measured by air-drying and re-weighing of the residual amount of added leaf litter and (4) changes in live body weight of the worms in each unit.

Copper was added as copper chloride, unless otherwise mentioned. The concentration in soil (< 2 mm) was measured by digesting 1-g samples of dried and ground soil in 10 ml of 1:1 nitric/sulphuric acid mix and bringing to a 100 ml volume with deionised water. Cu was determined by atomic absorption spectrophotometry in an air/acetylene flame at 324·7 nm wavelength.

In one experiment, the effect of copper was investigated at concentrations of 14 (unamended soil), 54, 131 and 372 mg Cu kg^{-1}. Observations were made at 6 weeks and the experiment performed in eight replicates. Adult worms were used of an average live weight of 1550 mg and varying between 1060 and 2156 mg.

In a subsequent experiment, the effect of a selected concentration of 160 mg Cu kg^{-1} was studied at three different temperatures, 12, 15 and 18 °C, with observations at 2, 4 and 6 weeks. The experiment was conducted in six replicates, using worms of an average weight of 1570 mg with a variation between 1400 and 1800 mg. The relative air humidity was kept constant at 78% at all tested temperatures. Loss of soil moisture content in this experiment remained limited to less than 2%, measured in units at 6 weeks and 18 °C.

The influence of soil pH was studied in another experiment in which the above-described sandy soil was treated with $Ca(OH)_2$ over a 14-day equilibration period to adjust its pH (KCl) from 4·8 (range 4·7–4·9) to 7·1

(range 7·1–7·2), or to an intermediate level of 6·0 (range 5·9–6·1). Copper was added as copper sulphate and investigated at concentrations of 14 (unamended soil), 83, 148 and 278 mg Cu kg^{-1}. Observations were made at 2·5 weeks. The experiment was done in five replicates with worms of an average weight of 1260 mg with a variation between 1025 and 1500 mg.

For comparative purposes the effect of copper was additionally measured in a calcareous sandy loam soil of pH 7·3. This soil type consisted of 17 % clay, 74 % silt, 3·4 % organic matter, 5·5 % $CaCO_3$, and had a soil moisture content of 35 %. Copper was investigated at a similar range as investigated in the corresponding experiment using sandy soil, *viz.* at concentrations of 13 (unamended soil), 63, 136 and 373 mg Cu kg^{-1}. Observations were made at 6 weeks. The experiment was performed in eight replicates using worms of an average weight of 1555 mg with a variation between 1050 and 2300 mg.

All experiments were set up according to a factorial design in complete blocks in which the initial weight of the worms at the beginning of the experiment was taken as a concomitant variable (Cox, 1958). Per block, the weights of the worms were selected in such a way as to minimise the variability between treatments. The data were statistically evaluated by analysis of (co)variance. Significant differences were determined either by computing the least significant difference among means (Sokal & Rohlf, 1969) or by applying the Newman-Keuls multiple range test (Steel & Torrie, 1960).

RESULTS

Effect in sandy soil

Table 1 shows the results of observations in units of *L. rubellus* at 6 weeks in Cu-amended sandy soil. Mortality was virtually absent, both among controls and among worms exposed to copper at concentrations up to 131 mg Cu kg^{-1}. Cocoon production and litter breakdown activity were significantly reduced at 131 mg Cu kg^{-1}. Copper had to reach a higher concentration of 372 mg Cu kg^{-1} before body weight gain differed significantly from controls.

Influence of temperature

The effect of a concentration of 160 mg Cu kg^{-1} in sandy soil was investigated further at different temperature conditions and exposure

TABLE 1
Effect of Copper on *L. rubellus* at Six Weeks. Average Values (±SD) of Eight Replicate Units, Each Unit Consisting of Five Worms in Sandy Soil

	Cu concentration (mg kg^{-1} soil)			
	*14**	*54*	*131*	*372*
Mortality (%) ($N = 40$)	0	7·5	2·5	17·5
Number of cocoons	70·6 ± 12·4[a]	62·1 ± 14·0[a]	41·0 ± 11·8[b]	3·3 ± 4·1[c]
Litter breakdown (g)	18·37 ± 0·96[a]	16·90 ± 2·20[a]	14·81 ± 2·07[b]	7·27 ± 2·76[c]
Body weight gain (%)	127·7 ± 21·1[a]	118·7 ± 10·9[a]	119·8 ± 19·0[a]	103·8 ± 15·3[b]

* Unamended soil.
[a,b,c] Any two means within a row not marked with the same letter were significantly different ($p < 0·05$).

durations. Table 2 shows that, during the observation period of 6 weeks, mortality remained absent among controls exposed at 12 and 15 °C, but was slightly higher at 18 °C. In Cu-amended soil, mortality was somewhat greater but its occurrence was not significant when compared with binomial distributions.

Cocoon production and litter breakdown activity tended to increase with increasing temperature, whereas body weight gain decreased with increasing temperature. After 4 weeks, however, litter breakdown activity showed a distinct optimum at a temperature of 15 °C.

Figure 1 illustrates the reducing effect of copper on body weight gain, cocoon production, and litter breakdown activity at different temperatures and exposure durations. Final body weights, expressed as a percentage of control weights, showed the greatest reduction at the lowest temperature. Generally, differences with controls tended to be more significant at shorter exposure duration and lower temperature. This could be partly attributed to the reduced variability of the measured variable (Table 2).

The positive influence of temperature on cocoon production and litter breakdown activity remained present with worms in Cu-amended soil (Table 2). Also, the optimum temperature of 15 °C for litter breakdown activity, observed at 6 weeks in unamended soil, remained unaltered in Cu-amended soil. As shown in Fig. 1, the effect of copper on litter breakdown activity at 6 weeks was less great at 15 °C than at either 12 or 18 °C, reflecting the more optimal condition at 15 °C.

TABLE 2

Influence of Temperature and Exposure Duration on the Effect of Copper (160 mg Cu kg^{-1}) on *L. rubellus*. Average Values (±SD) of Six Replicate Units, Each Unit Consisting of Five Worms in Sandy Soil

	Temperature (°C)	*Unamended soil* 2 weeks	4 weeks	6 weeks	*Cu treated soil* 2 weeks	4 weeks	6 weeks
Mortality (%) ($N = 30$)	12	0	0	0	0	13	17
	15	0	0	0	7	17	13
	18	0	13	10	13	17	37
Number of cocoons	12	$14{\cdot}2 \pm 1{\cdot}5^{a}$	$31{\cdot}6 \pm 4{\cdot}1^{a}$	$55{\cdot}2 \pm 8{\cdot}5^{a}$	$0{\cdot}5 \pm 0{\cdot}2^{a}$	$0{\cdot}3 \pm 0{\cdot}5^{a}$	$6{\cdot}5 \pm 4{\cdot}2^{a}$
	15	$20{\cdot}5 \pm 3{\cdot}3^{b}$	$42{\cdot}0 \pm 11{\cdot}5^{a}$	$91{\cdot}3 \pm 10{\cdot}3^{b}$	$0{\cdot}5 \pm 0{\cdot}5^{a}$	$2{\cdot}8 \pm 3{\cdot}6^{b}$	$18{\cdot}2 \pm 7{\cdot}9^{b}$
	18	$25{\cdot}7 \pm 4{\cdot}6^{c}$	$61{\cdot}2 \pm 19{\cdot}2^{b}$	$83{\cdot}8 \pm 11{\cdot}5^{b}$	$1{\cdot}5 \pm 1{\cdot}2^{a}$	$11{\cdot}2 \pm 8{\cdot}0^{c}$	$31{\cdot}4 \pm 8{\cdot}6^{c}$
Litter breakdown (g)	12	$2{\cdot}73 \pm 0{\cdot}22^{a}$	$3{\cdot}92 \pm 0{\cdot}46^{a}$	$9{\cdot}18 \pm 1{\cdot}94^{a}$	$0{\cdot}50 \pm 0{\cdot}19^{a}$	$0{\cdot}33 \pm 0{\cdot}35^{a}$	$3{\cdot}07 \pm 1{\cdot}14^{a}$
	15	$2{\cdot}53 \pm 0{\cdot}32^{ab}$	$5{\cdot}11 \pm 0{\cdot}99^{a}$	$14{\cdot}58 \pm 1{\cdot}27^{b}$	$1{\cdot}00 \pm 0{\cdot}30^{b}$	$2{\cdot}52 \pm 0{\cdot}85^{b}$	$12{\cdot}53 \pm 3{\cdot}14^{b}$
	18	$3{\cdot}20 \pm 0{\cdot}58^{b}$	$5{\cdot}78 \pm 1{\cdot}90^{a}$	$8{\cdot}68 \pm 0{\cdot}37^{a}$	$1{\cdot}18 \pm 0{\cdot}14^{a}$	$2{\cdot}79 \pm 1{\cdot}03^{b}$	$5{\cdot}32 \pm 0{\cdot}89^{a}$
Body weight gain (%)	12	$112{\cdot}3 \pm 2{\cdot}4^{a}$	$114{\cdot}2 \pm 5{\cdot}6^{a}$	$110{\cdot}2 \pm 8{\cdot}4^{a}$	$78{\cdot}4 \pm 3{\cdot}8^{a}$	$60{\cdot}5 \pm 9{\cdot}7^{a}$	$68{\cdot}8 \pm 9{\cdot}3^{a}$
	15	$112{\cdot}9 \pm 5{\cdot}5^{a}$	$99{\cdot}6 \pm 7{\cdot}8^{a}$	$101{\cdot}7 \pm 6{\cdot}4^{a}$	$80{\cdot}6 \pm 4{\cdot}6^{a}$	$71{\cdot}1 \pm 6{\cdot}4^{a}$	$78{\cdot}4 \pm 14{\cdot}6^{a}$
	18	$97{\cdot}5 \pm 3{\cdot}7^{b}$	$84{\cdot}2 \pm 10{\cdot}4^{b}$	$78{\cdot}5 \pm 8{\cdot}6^{b}$	$76{\cdot}6 \pm 5{\cdot}3^{a}$	$66{\cdot}4 \pm 5{\cdot}7^{a}$	$67{\cdot}0 \pm 7{\cdot}2^{a}$

[a,b,c] Any two means within a column for each parameter not marked with the same letter were significantly different ($p < 0{\cdot}05$).

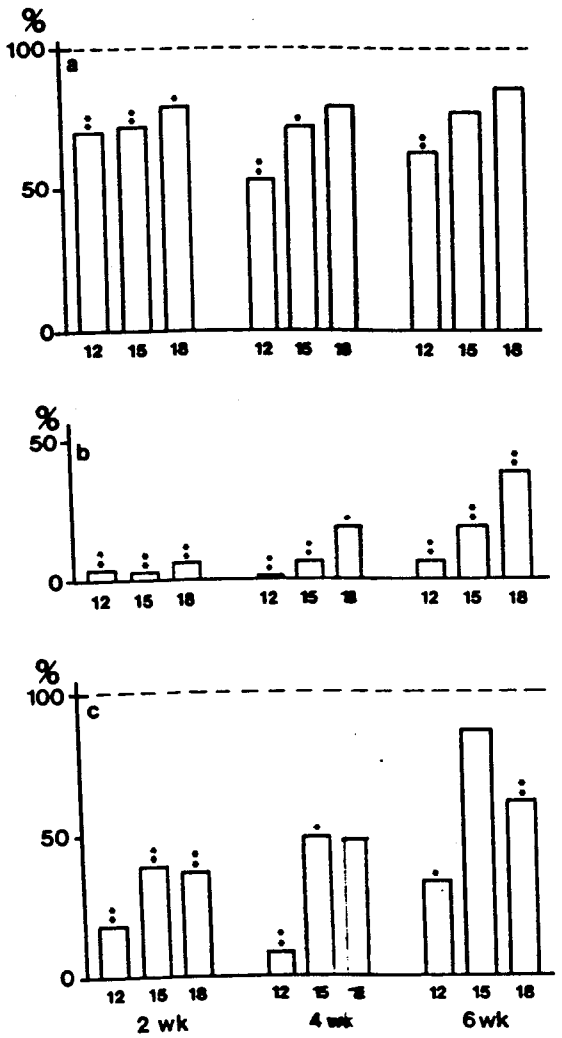

Fig. 1. The mean body weight (a), cocoon production (b) and litter breakdown activity (c) in units of *L. rubellus* ($N = 6$ units) exposed to Cu-amended (copper chloride) sandy soil (160 mg Cu kg^{-1}), expressed as the percentage of the control means of worms in unamended soil. Treatment means that were significantly different from control means are marked: $^{**}p < 0{\cdot}01$, $^{*}p < 0{\cdot}05$.

Influence of soil pH

The effect of pH neutralisation of sandy soil on copper toxicity is shown in Table 3. As expected, virtually no mortality occurred even at the highest Cu concentration tested (278 mg kg^{-1}).

Adjustment of pH from 4·8 to 7·1 did not have any positive effect on the earthworms in unamended soil. The activity of the earthworms even tended to decline in the base-adjusted soil, which was significant with regard to body weight gain. In Cu-amended soil, however, neutralisation of pH did show a favourable influence with regard to copper toxicity. Thus, the reduction of body weight gain, as caused by copper at a concentration of 278 mg kg^{-1}, was eliminated in soil with pH adjusted to 6·0 or 7·1. A similar effect was present with respect to litter breakdown activity and cocoon production. The interaction between the effect of copper and soil pH is illustrated in Fig. 2. It appeared that the adverse effect of copper on cocoon production was attenuated at pH 6·0 and completely eliminated at pH 7·1.

TABLE 3

Influence of Soil pH on the Effect of Copper on *L. rubellus*. Average Values (±SD) of Five Replicate Units, Each Unit Consisting of Five Worms in Sandy Soil

	pH	*Cu concentration (mg kg^{-1} soil)*			
		*14**	*83*	*148*	*278*
Mortality (%) ($N = 25$)	4·8	0	0	0	0
	6·0	4	0	0	0
	7·1	0	4	0	0
Number of cocoons	4·8	$53·8 \pm 9·2^{a}$	$51·4 \pm 6·2^{a}$	$39·6 \pm 15·2^{a}$	$26·8 \pm 14·5^{a}$
	6·0	$58·2 \pm 15·0^{a}$	$50·0 \pm 8·0^{a}$	$48·8 \pm 5·1^{a}$	$39·2 \pm 7·5^{a,b}$
	7·1	$44·4 \pm 10·0^{a}$	$47·7 \pm 5·5^{a}$	$40·7 \pm 15·2^{a}$	$48·2 \pm 9·3^{b}$
Litter breakdown	4·8	$7·46 \pm 1·51^{a}$	$6·24 \pm 1·22^{a}$	$6·02 \pm 1·96^{a}$	$4·90 \pm 1·01^{a}$
	6·0	$5·98 \pm 1·16^{a}$	$6·10 \pm 0·85^{a}$	$7·22 \pm 1·95^{a}$	$7·10 \pm 1·25^{b}$
	7·1	$6·18 \pm 1·45^{a}$	$5·74 \pm 1·49^{a}$	$4·82 \pm 2·11^{a}$	$6·96 \pm 1·64^{b}$
Body weight gain (%)	4·8	$145·4 \pm 5·7^{a}$	$141·8 \pm 8·4^{a}$	$130·7 \pm 8·0^{a}$	$17·8 \pm 9·6^{a}$
	6·0	$121·7 \pm 3·9^{b}$	$129·2 \pm 9·2^{a}$	$136·5 \pm 8·2^{a}$	$125·6 \pm 7·2^{b}$
	7·1	$122·2 \pm 7·1^{b}$	$125·8 \pm 13·9^{a}$	$125·8 \pm 7·5^{a}$	$128·2 \pm 6·1^{b}$

* Unamended soil.

[a,b,c] Any two means within a column for each parameter not marked with the same letter were significantly different ($p < 0·05$).

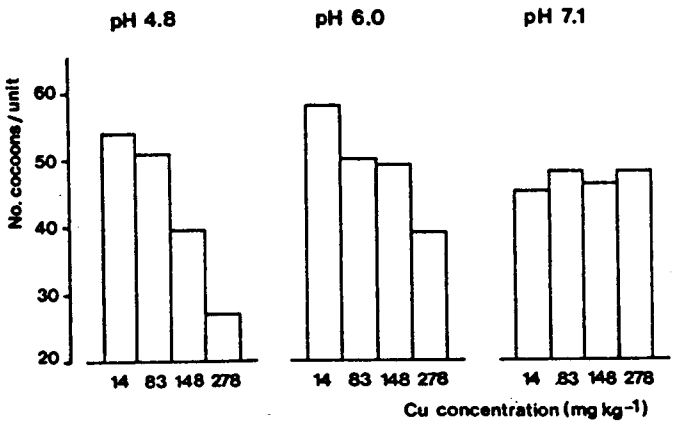

Fig. 2. The effect of copper, added as copper sulphate, on cocoon production at 2·5 weeks in sandy soil with soil pH adjusted from 4·8 to 7·1.

Effect in loam soil

To compare the effect of copper in a base-adjusted sandy soil with that in a naturally non-acid soil, an additional experiment was conducted using a calcareous sandy loam (5·5% $CaCO_3$). Contrary to what may be expected, copper added to this soil appeared to be as toxic as in sandy soil with unadjusted pH (Table 4). Litter breakdown activity was significantly reduced at 136 mg Cu kg^{-1} and cocoon production at 63 mg Cu kg^{-1}.

TABLE 4
Effect of Copper on *L. rubellus* at Six Weeks. Average Values (±SD) of Eight Replicate Units, Each Unit Consisting of Five Worms in Sandy Loam Soil

	Cu concentration (mg kg⁻¹)			
	*13**	*63*	*136*	*373*
Mortality (%) ($N = 40$)	0	5	2·5	17·5
Number of cocoons	$27·4 \pm 10·2^a$	$16·3 \pm 8·0^b$	$10·6 \pm 5·8^b$	$0·9 \pm 1·2^c$
Litter breakdown (g)	$10·54 \pm 1·78^a$	$9·15 \pm 2·24^a$	$8·11 \pm 1·42^b$	$6·80 \pm 1·09^b$
Body weight gain (%)	$109·6 \pm 15·0^a$	$99·8 \pm 13·6^a$	$103·4 \pm 17·2^a$	$95·8 \pm 20·1^a$

* Unamended soil.
[a,b,c] Any two means within a row not marked with the same letter were significantly different ($p < 0·05$).

Body weight gain, however, was not significantly reduced even at 373 mg kg^{-1}. Comparison of the general level of activity showed that the worms were considerably less active in sandy loam than in sandy soil.

DISCUSSION

L. rubellus is a ubiquitous soil-dwelling earthworm species, which has been described as a typical consumer of material rich in relatively undecomposed plant remains, including root fragments (Piearce, 1978). The litter-feeding habit of *L. rubellus* has been utilised in the present study as a behavioural test parameter of earthworm activity. Air-dried alder leaves are a suitable natural food substrate to this species (Nicholson & Owen, 1982), and, as shown in the present study, can be conveniently used for measuring litter breakdown activity.

Another test parameter of potential use in ecotoxicity studies is provided by the rate of cocoon production of *L. rubellus*. Cocoon production was found to respond more sensitively to the adverse effect of copper than either litter breakdown activity or body weight gain. Body weight gain generally appeared as the least sensitive test parameter, its reduction generally coinciding with the first occurrence of mortality.

According to the present results, copper inhibited growth at 6 weeks at a concentration of 370 mg kg^{-1} (sandy soil) or higher (sandy loam). Litter breakdown activity was significantly reduced at 130 mg kg^{-1}, in either sandy soil or loam soil, and cocoon production was inhibited at 130 mg kg^{-1} in sandy soil, and at 65 mg kg^{-1} in sandy loam. The results further suggest that significant sublethal toxic effects can be detected among worms exposed for a period as short as 2 weeks.

Studies with the compost worm *E. foetida* have shown that copper had to reach 500 mg kg^{-1} before growth at 8 weeks was significantly inhibited, and to 2000 mg kg^{-1} before cocoon production was reduced (Malecki *et al.*, 1982). However, it is unlikely that copper would occur in a similar chemical form in the horse manure used as a test medium for *E. foetida*, as in the agricultural soils employed in the present study. Therefore, it is not possible to draw any clear-cut conclusions from the present data about differences in intrinsic tolerance to copper between the two species.

The present results indicate that the optimum environmental temperature for *L. rubellus* is at 15 °C, when judged from the litter breakdown activity. Body weight gain and survival tended to be lower at

18 °C than at 12 or 15 °C. Cocoon production, however, increased with temperature increasing from 12 to 18 °C. These observations are in general agreement with those of Graff (1953), who stated that the optimum temperature of growth and development in this species is at 15 to 18 °C. When tested at an effective sublethal concentration, copper did not appear to cause any specific alteration of the temperature response, but merely tended to reduce the general performance of the earthworms.

The sublethal toxicity of copper in sandy soil (pH 4·8) was eliminated by adjusting the pH from 4·8 to 7·1. By itself, however, pH adjustment had a negative, rather than a beneficial, effect on the earthworms. Again, cocoon production proved to be the most sensitively responding test parameter, which, unlike growth and litter breakdown activity, continued to show decreases when copper was applied to soil whose pH had been adjusted to 6·0. Copper in this experiment was slightly less toxic than measured in the other experiments, probably because, in this particular experiment, the metal was added as copper sulphate instead of as copper chloride. Copper sulphate has a somewhat lower solubility in water than copper chloride.

The described influence of pH adjustment on copper toxicity in sandy soil may be related to a decrease in free cupric ion concentration with increasing pH in the soil solution (Sanders, 1983). However, when applied to a calcareous (5·5 % $CaCO_3$) sandy loam (pH 7·3) the toxicity appeared unattenuated. It is apparent that the factors determining copper toxicity may be complex and, in addition, are not necessarily limited to abiotic soil conditions determining the physico-chemical copper activity in soil. For instance, the behaviour of *L. rubellus* may be different in sandy soil than in loam soil which could affect the sensitivity to toxic substances in soil. Thus, *L. rubellus* has been consistently found to show a lower activity in loam soil than in sandy soil (Ma, details to be published later).

The present results suggest that *L. rubellus* may be suitably used in laboratory studies designed to evaluate the potential ecological impact of environmental pollutants in soil. In such tests, the reduction of litter breakdown activity and cocoon production would provide the most sensitively responding parameters of sublethal toxicity. Toxicity data referring to the impairment of vital functions of organisms have a greater ecological relevance and are of a better interpretability in ecotoxicological terms than data obtained from measurements of acute toxicity. This appears also from the present study which shows that the concentration range at which copper affects vital functions in *L. rubellus* coincides with

the range at which detrimental effects have been observed on earthworm populations in the field (see Introduction). However, further assessment studies will be necessary to establish the general value of the present laboratory test method in predicting effects in the field.

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