ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY **37,** 125–130 (1997)
ARTICLE NO. ES971536

Effects of Temperature on the Relative Toxicities of Cd, Cu, Pb, and Zn to *Folsomia candida* (Collembola)

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Received July 7, 1996

EC_{50}s for cadmium, copper, lead, and zinc were determined for juvenile production of *Folsomia candida* Willem, 1902, at 25, 20, and 15°C in a standard laboratory test system. Juvenile production of *F. candida* was too low at 25°C for reliable $EC_{50\text{-reproduction}}$ values to be determined. The $EC_{50\text{-reproduction}}$ values (μg g^{-1}) for cadmium, copper, and zinc were similar at both 20 and 15°C (20°C: Cd, 590; Cu, 700; Zn, 900; 15°C: Cd, 540; Cu, 640; Zn, 590). Corresponding values for lead were considerably higher (20°C: Pb, 2790; 15°C: Pb, 1570). In aerially contaminated field sites adjacent to primary zinc smelters, zinc is invariably present in surface soils at concentrations of at least 50 times those of cadmium. The similarity of the $EC_{50\text{-reproduction}}$ values for cadmium and zinc in *F. candida* at 20 and 15°C determined in this study strongly suggests that deleterious effects of mixtures of these metals on populations of Collembola in such sites can be attributed to zinc rather than cadmium.

INTRODUCTION

In a previous paper on the effects of manipulating standard ecotoxicological test conditions, it was found that the relative toxicities of cadmium, copper, lead, and zinc were similar for the ''standard'' springtail *Folsomia candida* Willem, 1902, in OECD artificial soil at pH 4.5, 5.0, and 6.0 at 20°C (Sandifer and Hopkin, 1996). In this paper, the effects of three different temperatures (15, 20, and 25°C) on the toxicities of cadmium, copper, lead, and zinc to *F. candida* have been examined in the standard laboratory test system at constant pH (6.0). The aims of the study were (a) to establish whether the metals were more toxic at a higher or lower temperature than that stipulated in the standard test protocol (20°C) (Riepert, 1993) and (b) to determine whether the relative toxicities of cadmium, copper, lead, and zinc were affected.

MATERIALS AND METHODS

Artificial soil was used as described in the OECD standard earthworm test, Guideline 207 (OECD, 1984), and the draft recommendation for the *F. candida* standard test (Riepert, 1993). The medium consisted (by dry weight) of 70% sand, 20% clay (kaolin clay), and 10% organic matter as *Sphagnum* peat. For further details, see Spurgeon *et al.* (1994). The pH of the medium was adjusted to 6.0 ± 0.05 at the start of the experiment with powdered calcium carbonate. The constituents for the artificial soil were air-dried, mixed thoroughly, and weighed into plastic boxes (275 × 155 × 95 mm). Solutions of cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$), copper nitrate ($Cu(NO_3)_2 \cdot 3H_2O$), lead nitrate ($Pb(NO_3)_2$), and zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$) (BDH chemicals, Poole, Dorset, UK) were mixed with the dry constituents to give the required percentage water content (ca. 30%) and metal concentrations in the soils. The same volume of distilled water was added to the controls. The concentrations of metals used in the 20°C test (in μg metal g^{-1} dry weight of soil) were 5, 20, 80, 300, and 1200 (cadmium); 10, 40, 200, 1000, and 3000 (copper); 100, 400, 2000, 10,000, and 50,000 (lead); and 100, 190, 350, 620, and 1200 (zinc). At both 15 and 25°C, the same concentrations were used for cadmium, copper, and lead, but for zinc, the concentrations 100, 300, 1000, 3000, and 10,000 were employed since some reproduction was still observed at 1200 μg Zn g^{-1} at 20°C. Consequently, an additional experiment was carried out at 20°C with zinc to confirm the lack of reproduction at and above 3000 μg Zn g^{-1}. Nitric acid digests of samples of contaminated soils were analyzed by atomic absorption spectrometry (for further details see Hopkin, 1989). Concentrations of metals were within 10% of the nominal values in every case. After equilibration for 48 hr, soils were added to 200-ml plastic vending machine cups (30 g into each, four replicates for each concentration) and 5 mg of dried yeast was placed in each as a food source for the Collembola.

Cultures of *F. candida* (Willem) were maintained in the laboratory at 20 ± 1°C under constant light, in plastic containers with a base of plaster of Paris mixed with graphite powder. A small amount of dried yeast was added weekly as a food source. All the *F. candida* used in the test were members of a ''Reading strain,'' derived from a single female isolated from a culture donated by Dr. J. Wiles of Southampton University. Ten adult springtails of equal size were added to each container using a pooter. The lid of a petri dish was lightly sprayed with distilled water and placed over the top of each cup to maintain high humidity in the containers. The tests were carried out at

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0147-6513/97 $25.00

15, 20, and 25 ± 1°C under constant light conditions. The petri dish lids were sprayed every 48 hr with distilled water and another 5 mg of yeast was placed in each experimental container after 2 weeks.

After 4 weeks the 20 and 25°C containers were flooded with distilled water and photographed individually from above on Fujichrome Provia color transparency slide film. Those from 15°C were left for 6 weeks as preliminary trials had revealed that an additional 2 weeks was required for the level of juvenile production to approach that of the controls at 20°C. The transparencies were projected onto a desktop viewer and the numbers of juveniles produced and adults surviving in each container were counted. EC_{50} values for reproduction (the concentration of metal at which juvenile production was reduced to 50% of that of the controls) were determined for each metal treatment at each temperature from the graphs (Figs. 1 and 2). Student *t* tests were performed to determine the significance of differences in the responses of *F. candida* between control and metal-treated soils at the three temperatures (Tables 1–3).

RESULTS

Juvenile production was very low in controls and in all metal treatments at 25°C (Table 1) and it was not possible to calculate reliable $EC_{50\text{-reproduction}}$ values for *F. candida* at this temperature. Thus, only the results for 20 and 15°C will be examined in detail here (Tables 2 and 3; Figs. 1 and 2).

Cadmium

A clear reduction in reproduction was observed at 1200 $\mu g\ g^{-1}$ at 20°C and 1000 $\mu g\ g^{-1}$ at 15°C whereas at 300 $\mu g\ g^{-1}$ at both temperatures there was little effect. There was also a significant reduction in juvenile production at 20 $\mu g\ g^{-1}$ at 15°C (Table 3), but this appears to be an isolated result as the values for 80 and 300 $\mu g\ g^{-1}$ were not significantly different from those of the control. Adult survival was not affected at 20°C (Table 2; Fig. 1A) but at 15°C it was significantly reduced at the highest concentration (Table 3; Fig. 2A). $EC_{50\text{-reproduction}}$ values were very similar at both temperatures (Table 4).

Copper

There was no reproduction at soil concentrations of 3000 $\mu g\ g^{-1}$ at 20 and 15°C and at both these temperatures juvenile numbers were significantly lower than those of controls at 1000 $\mu g\ g^{-1}$ (Tables 2 and 3; Figs. 1B and 2B). A significant reduction in adult survival also occurred at the highest concen-

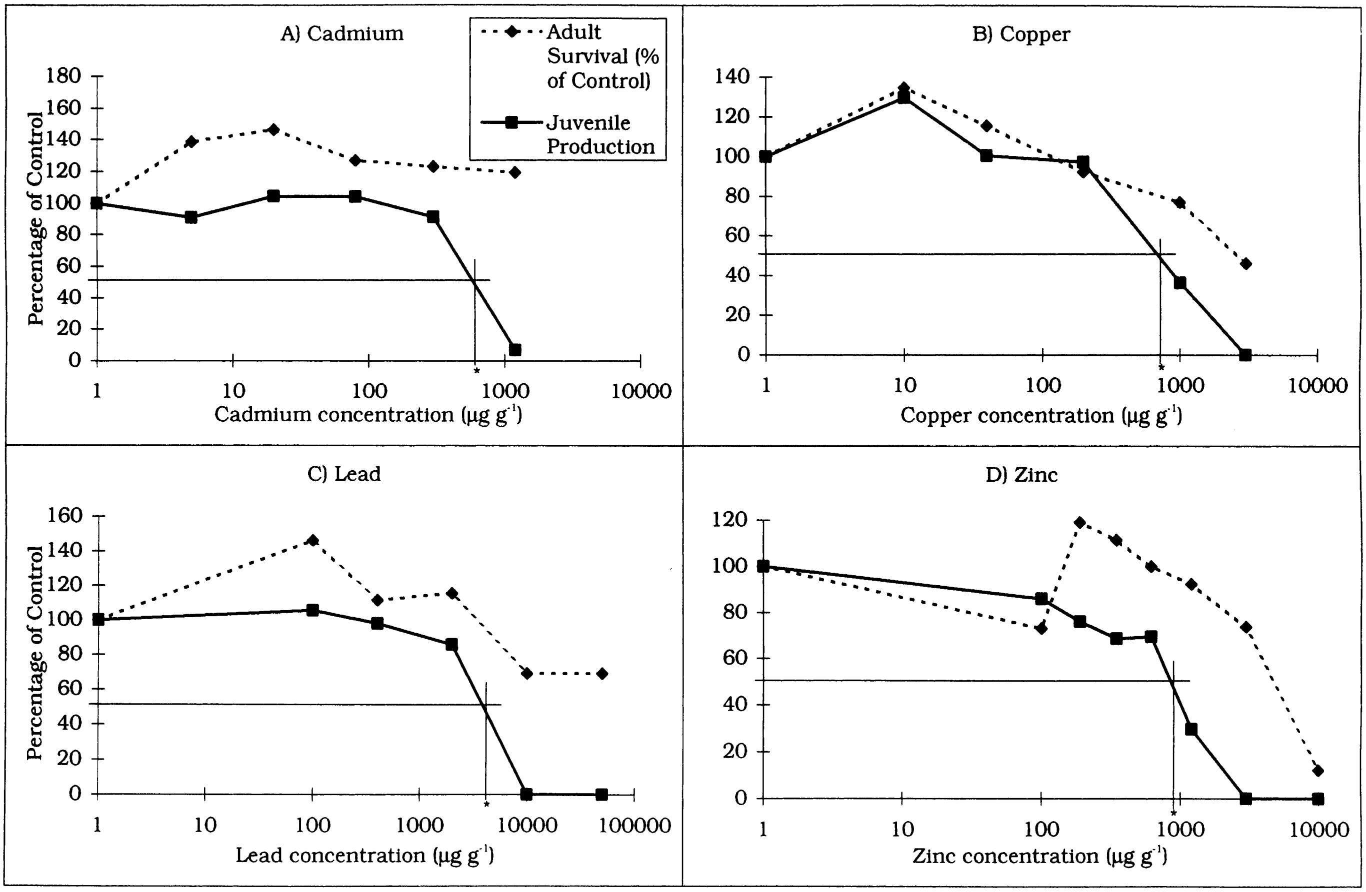

FIG. 1. Adult survival and juvenile production at 20°C expressed as a percentage of that of the control. *$EC_{50\text{-reproduction}}$ values.

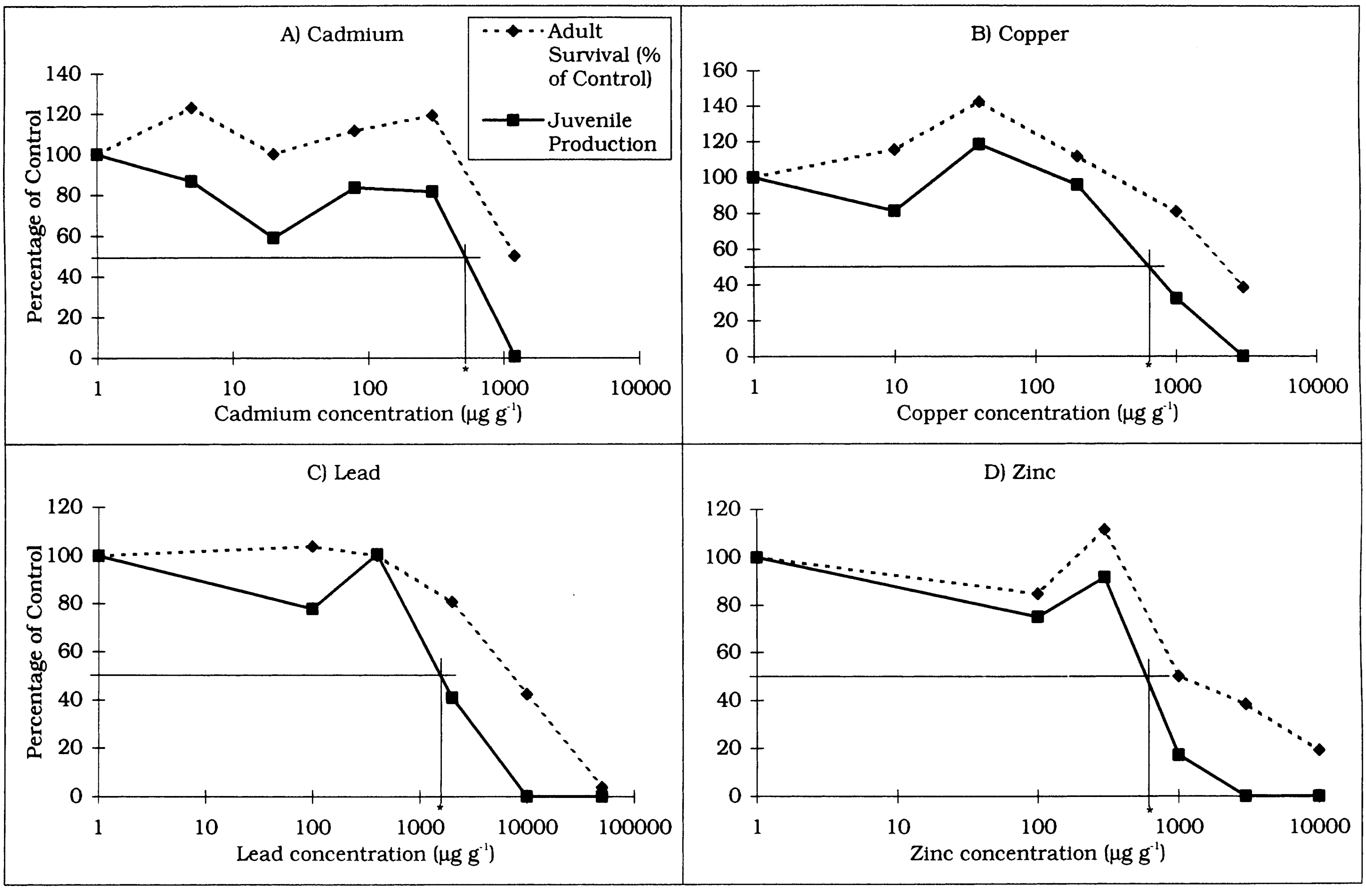

FIG. 2. Adult survival and juvenile production at 15°C expressed as a percentage of that of the control. $^{*}EC_{50\text{-reproduction}}$ values.

trations at 15°C (Table 3). $EC_{50\text{-reproduction}}$ values were in close agreement at 20 and 15°C (Table 4).

Lead

At 20°C, no reproduction occurred at or above soil concentrations of 10,000 $\mu g\ g^{-1}$ (Table 2; Fig. 1C). At 15°C, a significant reduction occurred at 2000 $\mu g\ g^{-1}$ (Table 3; Fig. 2C). At 20°C, the $EC_{50\text{-reproduction}}$ value was about twice that at 15°C (Table 4).

Zinc

There was no reproduction at or above 3000 $\mu g\ g^{-1}$ at both temperatures and significantly reduced reproduction at 1200 $\mu g\ g^{-1}$ at 20°C and 1000 $\mu g\ g^{-1}$ at 15°C (Tables 2 and 3; Figs. 1D and 2D). Adult survival was significantly reduced at and above 1000 $\mu g\ g^{-1}$ at 15°C (Table 3), but at 20°C this only occurred at the highest concentration (Table 2). $EC_{50\text{-reproduction}}$ values were similar at both temperatures (Table 4).

Effects of Temperature

At 15°C, the level of juvenile production in the control samples and the lowest metal concentrations was lower than that at 20°C (Tables 2 and 3). However, since EC_{50}s were calculated relative to the controls, it was legitimate to compare the relative toxicities of the metals at the two temperatures. The EC_{50} values suggest that at 15°C, reproduction in *F. candida* is generally more sensitive to all four metals than that at 20°C (Table 4).

Relative Toxicities of Metals

At 20 and 15°C, the $EC_{50\text{-reproduction}}$ values for cadmium, copper, and zinc in *F. candida* were similar, but lead was consistently less toxic at 20°C. For example, lead was three times less toxic than cadmium at 15°C and five times less toxic at 20°C (Table 5).

DISCUSSION

The results presented here indicate that at 25°C, juvenile production of *F. candida* in the standard test system is too low for the effects of chemicals on this species to be determined reliably. Snider and Butcher (1973) stated that ''26°C possibly approaches the upper limit of tolerance for *F. candida*.'' This view concurs with the work of Marshall and Kevan (1962) who found that eggs laid at 24°C did not hatch when transferred to 28°C. Previous work has demonstrated that altering ambient temperature affects reproduction in *F. candida.* Hutson (1978) reared this species at 15, 20, and 25°C in the laboratory. He

TABLE 1
Adult Survival and Juvenile Production (Mean ± Standard Errors; 4 Replicates at Each Concentration) for Each Metal at 25°C

Cadmium			Copper			Lead			Zinc		
Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production
0	8.0 (±0.4)	30 (±15)	0	8.0 (±0.4)	30 (±15)	0	8.0 (±0.4)	30 (±15)	0	8.0 (±0.4)	30 (±15)
5	6.8 (±0.9), ns	9 (±1), ns	10	7.0 (±1.2), ns	3 (±1), ns	100	6.0 (±1.7), ns	14 (±5), ns	100	8.8 (±1.9), ns	45 (±27), ns
20	6.8 (±1.1), ns	7 (±1), ns	40	8.0 (±0.9), ns	11 (±6), ns	400	8.0 (±1.4), ns	74 (±29), ns	300	8.0 (±0.9), ns	128 (±27)*
80	6.5 (±1.0), ns	23 (±14), ns	200	8.3 (±0.9), ns	36 (±19), ns	2000	8.0 (±0.7), ns	64 (±27), ns	1000	6.3 (±0.3), ns	68 (±18), ns
300	5.5 (±1.3), ns	32 (±9), ns	1000	7.5 (±0.3), ns	11 (±3), ns	10,000	6.3 (±0.9), ns	0.8 (±1)** ns	3000	7.0 (±0.6), ns	0*
1200	3.3 (±0.8)*	5 (±2), ns	3000	2.0 (±0.4)*	1 (±1), ns	50,000	0*	0**	10,000	2.0 (±0)*	0*

Note. ns, not significantly different from the control.
* Significantly different from the control at the 1% level.
** Significantly different from the control at the 5% level.

found that at 15 and 20°C there was little difference in the duration of the oviposition period (17.2 and 16.8 days, respectively), but at 25°C females laid over a mean period of just 6.4 days. Hutson (1978) also found that adult survival was similarly affected; at 15 and 20°C adults survived for in excess of 400 days, whereas at 25°C they only lived for a mean of 126 days. Snider and Butcher (1973) found a similar effect, mean life span being 240 days at 15°C, 136 days at 21°C, and 72 days at 26°C.

Egg production has also been found to decrease at higher temperatures. Snider and Butcher (1973) found that at 15°C it was common for between 160 and 200 ova to be laid in a batch at one time. At 21°C, the greatest number recorded was 157 ova and at 26°C it was only 44. The mean number of ovipositions per female was 13 at both 15 and 21°C and 5 at 26°C. Temperature has been found to affect egg viability as well. At 15 and 20°C viability was always greater than 95% but at 26°C it never exceeded 88% (Snider and Butcher, 1973). Thus, it is plain that at 25°C, in the laboratory at least, the reproductive ability and adult survival of *F. candida* is significantly reduced in comparison to those at 20 and 15°C.

There have been very few studies carried out to observe the effects of temperature on metal toxicities in Collembola. One particular study looked at *Orchesella cincta,* another springtail, and the ways in which temperature affects cadmium kinetics in this species (Janssen and Bergema, 1991). It was found that cadmium assimilation efficiency and excretion were both lower at 10°C (7.3% and 0.040 days^{-1}, respectively) than those at 20°C (11.3% and 0.202 days^{-1}, respectively), indicating that in *O. cincta,* the rate of cadmium excretion is inversely related to temperature. Work by Crommentuijn *et al.* (1993) has found an $EC_{50\text{-reproduction}}$ value for cadmium in *F. candida* of 227 μg Cd g^{-1} at 20°C and pH 6.0. While the figures presented in the present study are higher than this value, Crommentuijn *et al.* (1993) also found an LC_{50} value of 893 μg Cd g^{-1} which is not exceeded here.

Data reveal a general reduction in reproduction (Table 3) and the rate at which it occurs at 15°C (from preliminary trials). However, it is possible that if the samples were left for longer than 6 weeks, the numbers of juveniles produced may eventually have reached those of the controls at 20°C. There were no clear effects of temperature on the toxicities of cadmium, copper, and zinc (lead appears to be about twice as toxic at 15°C). While this paper indicates no reason why 20°C should not be used as the standard test temperature, previous work has found that slight advantages such as increased fecundity and longevity occur at 15°C (Hutson, 1978; Marshall and Kevan, 1962; Snider and Butcher, 1973). However, while it is important to bear in mind that 20°C may not be representative of field conditions where it may be colder (for example, in the UK and northern Europe), the results presented here do not demonstrate a notable difference between the two temperatures. Thus, it is not recommended that the test be carried out at 15°C as it does not produce results that are significantly different from those at 20°C and involves a minimum extension of the experimental period of 2 weeks.

TABLE 2
Adult Survival and Juvenile Production (Mean ± Standard Errors; 4 Replicates at Each Concentration) for Each Metal at 20°C

Cadmium			Copper			Lead			Zinc		
Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production
0	6.5 (±1.2)	797 (±95)	0	6.5 (±1.2)	797 (±95)	0	6.5 (±1.2)	797 (±95)	0	6.5 (±1.2)	797 (±95)
5	9.0 (±0.7), ns	724 (±78), ns	10	8.8 (±0.6), ns	1032 (±169), ns	100	9.5 (±0.3), ns	842 (±74), ns	100	4.8 (±0.5), ns	685 (±61), ns
20	9.5 (±0.5), ns	833 (±44), ns	40	7.5 (±1.2), ns	801 (±46), ns	400	7.3 (±1.2), ns	781 (±56), ns	190	7.8 (±0.9), ns	607 (±74), ns
80	8.3 (±0.8), ns	831 (±52), ns	200	6.0 (±0.6), ns	774 (±27), ns	2000	7.5 (±0.6), ns	685 (±93), ns	350	7.3 (±0.8), ns	548 (±57), ns
300	8.0 (±0.4), ns	826 (±42), ns	1000	5.0 (±0.8), ns	291 (±46)*	10,000	4.5 (±1.3), ns	0*	620	6.5 (±0.3), ns	554 (±50), ns
1200	7.8 (±0.6), ns	54 (±9)*	3000	3.0 (±1.5), ns	0*	50,000	4.5 (±0.6), ns	0*	1200	6.0 (±0.4), ns	237 (±35)*
									3000	4.8 (±1.1), ns	0 (±0)*
									10,000	0.8 (±0.5)*	0 (±0)*

Note. ns, not significantly different from the control.
* Significantly different from the control at the 1% level.

TABLE 3
Adult Survival and Juvenile Production (Mean ± Standard Errors; 4 Replicates at Each Concentration) for Each Metal at 15°C

Cadmium			Copper			Lead			Zinc		
Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production	Soil conc. (μg g^{-1})	Adult survival	Juvenile production
0	6.5 (±0.5)	492 (±52)	0	6.5 (±0.5)	492 (±52)	0	6.5 (±0.5)	492 (±52)	0	6.5 (±0.5)	492 (±52)
5	8.0 (±0.8), ns	427 (±57), ns	10	7.5 (±0.6), ns	399 (±36), ns	100	6.8 (±0.9), ns	383 (±45), ns	100	5.5 (±0.9), ns	369 (±53), ns
20	6.5 (±0.6), ns	290 (±12)*	40	9.3 (±1.7), ns	582 (±81), ns	400	6.5 (±0.9), ns	495 (±62), ns	300	7.3 (±1.3), ns	450 (±85), ns
80	7.3 (±1.1), ns	412 (±26), ns	200	7.3 (±0.5), ns	471 (±26), ns	2000	5.3 (±0.8), ns	201 (±53)**	1000	3.3 (±0.5)**	85 (±31)*
300	7.8 (±0.5), ns	402 (±53), ns	1000	5.3 (±1.0), ns	158 (±39)**	10,000	2.8 (±1.0)**	0*	3000	2.5 (±0.6)**	0*
1200	3.3 (±0.5)*	4.0 (±2.0)*	3000	2.5 (±0.9)*	0*	50,000	0.3 (±0.3)*	0*	10,000	1.3 (±0.6)*	0*

Note. ns, not significantly different from the control.
* Significantly different from the control at the 1% level.
** Significantly different from the control at the 5% level.

TABLE 4
$EC_{50\text{-reproduction}}$ Values in μg g^{-1} for Each Metal at 20 and 15°C Derived from the Graphs Presented in Figs. 1 and 2

	EC_{50}			
	Cadmium	Copper	Lead	Zinc
20°C	590	700	2970	900
15°C	540	640	1570	590

Whichever of the two temperatures (20 or 15°C) is used, the standard test seems to be appropriate for determining the relative toxicities of metals to *F. candida*, and possibly other species of Collembola (Hopkin, 1997). This information can then be used in attempts to identify the most toxic metal within a ‘‘cocktail’’ of elements. For example, in the vicinity of a primary cadmium, lead, and zinc smelting works in Avonmouth, South West England, the mean ratio of concentrations of Cd:Cu:Pb:Zn in surface soils is 1:7:50:93 (Spurgeon and Hopkin, 1995). The toxicities of cadmium, copper, and zinc to *F. candida* are similar in the laboratory (Tables 4 and 5). Thus, since zinc occurs in Avonmouth soils at more than 10 times the concentration of copper, and nearly 100 times that of cadmium, it is clear that the deleterious effects of metal pollution on soil Collembola are most likely to be due to zinc poisoning in this situation. This is clearly the case for woodlice (Hopkin and Hames, 1994; Drobne and Hopkin, 1995), snails (Laskowski and Hopkin, 1996a,b), and earthworms (Spurgeon and Hopkin, 1995; Spurgeon *et al.,* 1994), which are absent close to the Avonmouth smelter due to heavy zinc contamination.

CONCLUSIONS

1. A temperature of 25°C is too high for successful calculation of $EC_{50\text{-reproduction}}$ values for metals in the standard test with *F. candida.* The recommended temperature of 20°C is satisfactory, but 15°C extends the length of the test for at least 2 weeks.

2. Since the $EC_{50\text{-reproduction}}$ values for cadmium, copper, and zinc in *F. candida* are similar, this suggests that where mixtures of these elements are present in field soils, the metal with the highest concentration will be the one that limits the distribution of Collembola. In aerially contaminated soils adjacent to primary zinc smelters, zinc is invariably present at by far the highest levels and deleterious effects on Collembola can thus be attributed to this element rather than cadmium or copper.

TABLE 5
$EC_{50\text{-reproduction}}$ Values of Copper, Lead, and Zinc Relative to Cadmium at 20 and 15°C

	Cadmium	Copper	Lead	Zinc
20°C	1	1.2	5.0	1.5
15°C	1	1.2	2.9	1.1

Note. (EC_{50} for metal (μg g^{-1})/EC_{50} for cadmium (μg g^{-1})).

ACKNOWLEDGMENTS

This work was funded by an NERC Industry Targeted Studentship with additional support from National Power Plc, and the Institute of Terrestrial Ecology at Monks Wood. Aspects of the work were funded by a grant from the Leverhulme Trust.

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