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Influence of Temperature on the Toxicity of Zinc to the Earthworm *Eisenia fetida*

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A range of toxicity tests have been proposed to assess the potential hazards of pollutants to earthworms (Van Gestel and Van Straalen, 1994). Of these, the two acute toxicity tests using *Eisenia fetida* recommended by the OECD (1984) and EEC (1985) have become routinely used in the risk assessment and regulation of new and existing chemicals (Greig-Smith, 1992). In addition to the acute tests, procedures have also been proposed for measuring the sub-lethal effects of chemicals on parameter such as reproduction and weight change (Kokta, 1993; Van Gestel *et al.,* 1989). In both the lethal and sub-lethal toxicity tests developed with worms, attempts have been made to standardise test conditions to allow results from different laboratories to be directly compared. However, variability in exposure conditions and responses are fundamental to determine the effects of pollutants under natural conditions (Forbes and Depledge, 1992). In the field, conditions such as light, moisture availability, pH, temperature and humidity all fluctuate over time. Such variations affect both the sensitivity and exposure of individuals to toxic chemicals. Hence when evaluating the potential effects of pollutants, it may be important to know how changes in test conditions influence toxicity.

Temperature is one of the most important environmental conditions standardised in lethal and sub-lethal toxicity tests. However, little data are available on its influence on the responses of earthworms to toxicants. In the tests proposed by the OECD (1984), Kokta (1993) and Van Gestel et al. (1989) a standard temperature of 20°C is used to maximise growth and cocoon production, whilst maximising survival (Edwards and Bohlen, 1996; Viljoen *et al.*, 1992). In the field, earthworms can be exposed to toxicants at temperatures both above and below 20°C. Hence, in the present paper we have assessed the effects of different temperatures on the lethal and sub-lethal toxicity of zinc for the earthworm *Eisenia fetida.* Zinc was chosen for this work, since previous studies have indicated that this metal is most likely to be limiting the abundance of earthworms close to a smelter situated at Avonmouth in South-west England (Spurgeon and Hopkin, 1995; 1996a; Spurgeon *et al.,* 1994).

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MATERIALS AND METHODS

The effects of zinc on the survival and cocoon production of *Eisenia fetida* were measured at three temperatures 15, 20 and 25°C. All tests were conducted using the standardised reproduction toxicity test proposed by Van Gestel *et al. (1989).* This procedure uses an artificial soil medium consisting (by dry weight) of 70% sand, 20% kaolin clay and 10% organic matter (as *Sphagnum* peat), with pH adjusted to 6.0 ± 0.5 by the addition of powdered calcium carbonate (for further details, see OECD, 1984). The constituents for the artificial soil were air dried, mixed thoroughly, and weighed into plastic boxes (275 x 155 x 95 mm). For the zinc treated soils, solutions of zinc nitrate ($Zn\ N0_3.6H_20$) (BDH chemicals, Poole, Dorset, UK) were mixed with the dry constituents to give the required water content (35% wet weight) and metal concentrations in the test soil. Zinc concentrations of 190, 350, 620, 1200, 2000 µg Zn g^{-1} dry weight of soil were used in all tests. The same volume of distilled water was added to controls. Four replicates were used for each of the test concentrations and controls.

Eisenia fetida were obtained from a commercial supplier, where they had been reared in outdoor culture units. All worms were adult, fully clitellate and had a mean weight of 260 mg (190 - 480 mg). Prior to the experiment, the worms were maintained in uncontaminated artificial soil for one week at the same temperature as the test for which they were latter used. After this period, ten worms were added to each replicate. The containers were covered to prevent water loss and maintained at the relevant test temperature for 21 days in constant light. During the experiment, a small food pellet (3 g dry weight) of horse manure (collected from an animal that had been grazing uncontaminated pasture, and had not undergone any recent medication) was added weekly to each container to increase rates of cocoon production (Spurgeon and Hopkin, 1995; Van Gestel *et al.*, 1989).

For each toxicity test, effects on mortality and reproduction were measured. Mortality was assessed by counting the number of worms alive after 14 days. Reproduction was assessed by wet sieving the soil at the end of the experiment (21 days) using a 1 mm sieve and counting the number of cocoons present. The number of cocoons in each container was related to survivorship to allow cocoon production rates expressed as cocoon/worm/week to be calculated. LC_{50}s and EC_{50}s were determined from the mortality and cocoon production data by probit analysis and the linear interpolation technique (Norberg-King, 1993).

At the end of each experiment, eight worms (two from each replicate if available) were analysed for zinc concentration. Prior to preparation of the earthworms for digestion, worms were starved for 72 hours to remove any soil present in the gut. Worms were then dried to constant weight, digested in concentrated nitric acid and analysed for zinc content by flame atomic absorption spectrophotometry using a Varian Spectra AA-30 (see Hopkin, 1989).

RESULTS AND DISCUSSION

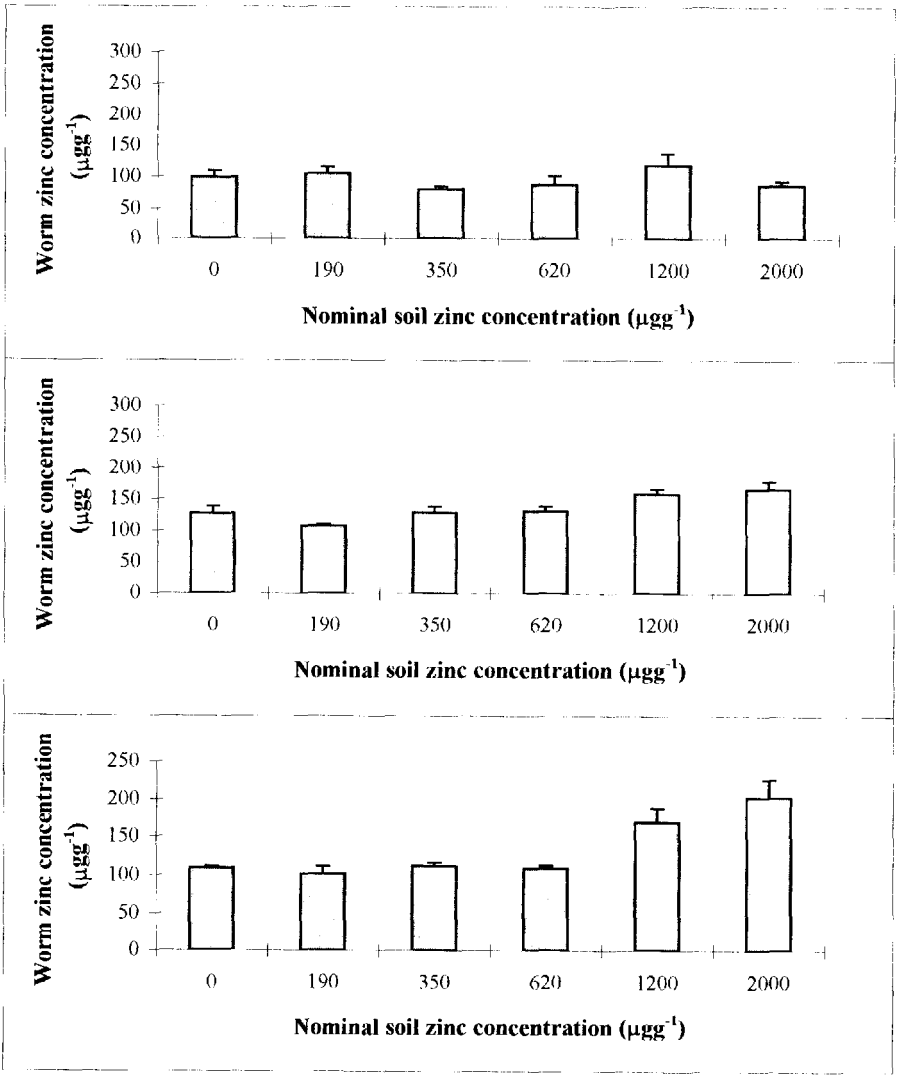
Survival at all test temperatures was significantly lower than controls at 2000 µg Zn g^{-1}. The LC_{50} values (with 95% confidence intervals) for survival were 1598 (1460 - 1760) µg Zn g^{-1} in the test conducted at 15°C, 1235 (811 - 2855) at 20°C and 1131 µg Zn g^{-1} at 25°C. Thus, the highest LC_{50} was for the test conducted at 15°C and the lowest for the 25°C test, suggesting an increase in zinc toxicity with temperature.

Table 1 Cocoon production rates of *Eisenia fetida* exposed to zinc in artificial soil using the protocol of Van Gestel *et al.* (1989). Asterisks indicate rates that are significantly different from controls at * P<0.05, ** P<0.01, *** P<0.001

Nominal zinc Concentration (µgg^{-1})	Cocoons\worm\week (+/- SE)		
	15°C	20°C	25°C
0	0.124	0.149	0.266
190	0.112	0.11	0.156 *
350	0.094	0.075	0.087 **
620	0.011 *	0.016 **	0.028 **
1200	0 ***	0 ***	0.12 ***
2000	0 ***	0 ***	0 ***

The cocoon production rates of control worms were highest at 25°C and lowest at 15°C (Table 1), although the control rates recorded in all three tests were well below the optimum for this species (Van Gestel *et al*, 1992). This was probably due to the small mean size of the worms used (mean weigh 260 mg compared to OECD (1984) recommended 300-600 mg).

As anticipated from the results of previous toxicity experiments with zinc (Spurgeon and Hopkin, 1995, 1996b; Spurgeon et al., 1994), cocoon production was more sensitive than mortality. For both the 15°C and 20°C test, no cocoons were collected at 1200 and 2000 µg Zn g^{-1} and significantly reduced cocoon production was found at 620 µg Zn g^{-1}. In the 25°C test, no cocoons were found in the 1200 and 2000 µg Zn g^{-1} soils and rates were significantly reduced at 190, 350 and 620 µg Zn g^{-1} (Table 1). The lowest EC_{50} of 234 µg Zn g^{-1} was for the 25°C. The value for the 20°C test was somewhat higher and was 308 µg Zn g^{-1}, while the highest value of 382 µg Zn g^{-1} was for the 15°C test. Thus, the lowest EC_{50}s were recorded at the higher test temperatures, suggesting that, as for the LC_{50}s, there is an increase in the toxicity of zinc at higher temperatures.



Figures 1a-c Mean tissue zinc concentration +/- SE for *Eisenia fetida* maintained in zinc amended artificial soil at a) 15°C, b) 20°C and c) 25°C. No significant differences in zinc concentration were found between any of the zinc exposed and controls worms at any test temperature.

Zinc accumulation in the tissues of exposed worms increased with temperature although there was no concentration at any temperature at which zinc levels were significantly increased above those found in control worms (Fig. 1a-c). Examples of increase in zinc levels are given for the worms in the 1200 µg Zn g^{-1} soil. Worms at 25°C had a mean zinc body content of 169 µg Zn g^{-1}, at 20°C mean worm zinc concentration was 159 µg Zn g^{-1}, while at 15°C the mean zinc level

was only 119 µg Zn g^{-1} (Fig. 1a-c). A two-factor analysis of variance using zinc concentration and temperature as the variable factors indicated a significant interaction between zinc concentration and test temperature($P < 0.001$). Significant effects of temperature and zinc treatment were also found. Thus, zinc burdens were dependent on both concentrations of zinc and the temperature to which the worms were exposed. The increase in zinc burdens with temperature indicates a greater exposure of earthworms, resulting in the higher toxicity found in this study.

Variations in the toxicity of chemicals at different temperatures could have profound effects on the impact of pollutants across a range of spatial and temporal scales. This is particularly true for terrestrial systems, which by their nature are more prone to temperature fluctuations than freshwater or marine environments. Little data are available in the literature concerning the relation between temperature and toxicity. The only direct evidence of a temperature mediated effect on the toxicity of a pollutant for soil invertebrates is given by Demon and Eijsackers (1985), who found an increased toxicity of azinphos-methyl and lindane for the isopod *Philoscia muscorum* at high temperatures. Greater toxicity was attributed to an increase in woodlouse metabolism resulting in more uptake at the highest temperature.

The increased toxicity of zinc to *Eisenia fetida* at high temperatures could also result from an alteration in metal kinetics favouring uptake. High temperatures have been shown to increase cadmium assimilation in the collembolan *Orchesella cincta*, the oribatid mite *Platynothrus peltifer* and the crab *Palaemon elegans* (Janssen and Bergema, 1991; Nugegoda and Rainbow, 1987). For *Orchesella cincta,* increased uptake was matched by increased excretion, resulting in similar body burdens at different temperatures, however, for *Platynothrus peltifer* elimination rate was not temperature dependent and elevated body burdens were found in animals maintained at high temperatures (Janssen and Bergema, 1991).

The effects of temperature on metal kinetics in earthworms have not been studied in laboratory tests. However, indirect evidence is given by seasonal measurements of metal burdens in contaminated soils. Bengtsson and Rundgren (1992) noted a reduction in lead levels of *Lumbricus terrestris* sampled during winter. This suggests an increase in elimination relative to uptake for lead under cold conditions. Morgan and Morgan (1993) also found fluctuations in the cadmium, lead and zinc burdens of *Lumbricus rubellus* through the year. However, in contrast to the results of Bengtsson and Rundgren (1992), highest concentrations were found predominately during the winter months. The variations in seasonal body burdens between these studies, suggest that metal levels are dependent on a range of co-variable factors such as population structure, age, migration and food availability as well as the metal kinetics of the exposed earthworm species (Janssen *et al,* 1990).

A further explanation for the greater toxicity of zinc at the highest test temperatures is that the volubility of the metal is increased. Any increase in metal solubility will raise exposure as the uptake of metals occurs primarily by passive adsorption of metal ions across the body wall (Spurgeon and Hopkin, 1996b). Hogg *et al. (1993)* found that the desorption of copper from spiked soils increased with temperature. Furthermore, Hooda and Alloway (1993, 1994) recorded an increase in the uptake of trace elements by ryegrass *(Lolium perenne)* grown in sludge contaminated soils at 25°C compared to plants at 15°C. DTPA-extractable metal concentrations generally supported the results of the plant analysis. In contrast to the above results suggesting an increase in metal availability at high temperature, Tong *et al,* (1995) found that the incubation of manganese spiked soils at high temperatures accelerated a decline in the DTPA-extractable metal levels, indicating lower solubility. Thus although some evidence exists indicating greater solubility of metals at high temperatures, data are not consistent between studies with different metals.

Literature data on the effects of temperature on metal kinetics in exposed animals and availability in soils are confusing making it difficult to determine which of these factors is primarily responsible for the increased toxicity of zinc found at the higher test temperatures used in this study. Further work is required if causal relationships are to be found that fully explain the interactions between temperature and the toxicity of metals for soil invertebrates.

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