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Effect of temperature and season on reproduction, neutral red retention and metallothionein responses of earthworms exposed to metals in field soils

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Laboratory and field studies demonstrate metal effects on earthworm life-cycle and biochemical responses are not influenced by temperature regime.

Abstract

This study investigated the short-term survival, reproduction and physiological (lysosomal membrane stability, metallothionein transcript copy number, body tissue metal concentrations) responses of *Lumbricus rubellus* exposed to metal contaminated field soils under different laboratory temperatures (10, 15 and 20 °C) and physiological responses of earthworms collected from the field in three different seasons (spring, autumn, winter). In the laboratory, metal contaminated soils had significant effects on reproduction ($p < 0.001$), metallothionein-2 (*MT-2*) expression ($p = 0.033$) and earthworm As ($p = 0.003$), Cd ($p = 0.001$), Pb ($p < 0.001$) and Zn ($p < 0.001$) concentration, but not lysosomal membrane stability and tissue Hg and Cu. No effect of temperature was found for any parameter. Principal component analysis of extractable and tissue metal concentrations indicated PC1 as a measure of metal stress. Both cocoon production ($r = -0.75$) and *MT-2* induction ($r = 0.41$) were correlated with PC1. A correlation was also found between cocoon production and *MT-2* expression ($r = -0.41$). Neutral red retention and *MT-2* measurements in worms collected from the field sites in three seasons confirmed the absence of a temperature effect on these responses.

Keywords: Combined stress; Cocoon production; Metallothionein; Lysosomal membrane stability; Temperature

1. Introduction

The responses of soil invertebrates to chemicals can differ depending on the prevailing environmental condition under which the species is exposed. While classically soil properties such as organic matter content, soil pH, soil clay content and cation exchange capacity are the main environmental conditions considered (Jager et al., 2003; Lock et al., 2000); prevailing climate may also affect biological responses. Because unlike soil parameters, temperature and rainfall can show short-term temporal variations, the effects of climate on chemical toxicity in the field can be more difficult to interpret. In a literature review of the effect of temperature on chemical toxicity for aquatic species, Heugens et al. (2001) found that generally, organisms living in conditions near to their environmental tolerance limits appeared to be more vulnerable to

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additional chemical stress. In the case of temperature, an increase was most frequently associated with a greater toxicity for single substances and mixtures.

While there is some information on the mechanisms of combined toxicity between chemicals in mixtures (Yang, 1994), relatively little is known about how chemical and non-chemical stressors may interact. It may be hypothesised that non-chemical stressors may change the physiology of organisms that may reveal themselves through effects on individual sensitivity (Parker et al., 1999). Indeed, for aquatic systems Heugens et al. (2001) explained the observed effects of temperature on toxicity in terms of several physical and physiological processes, such as bioavailability and toxicokinetics. This conclusion was based on a meta-analysis of the large volume of literature concerning the effects of temperature on the toxicity of single compounds to aquatic organism. In terrestrial species, the effects of temperature on single compound toxicity has been measured in a number of studies. These have show that temperature can increase toxicity (Demon and Eijsackers, 1985; Illmer and Mutschlechner, 2004; Spurgeon et al., 1997), reduce toxicity over time (Martikainen and Rantalainen, 1999) or have no directional effect: both for different static temperatures (Abdel-Lateif et al., 1998; Sandifer and Hopkin, 1997) and for different temperature regimes (Smit and VanGestel, 1997; Spurgeon et al., 2005). The growing acceptance that (eco)toxicology is merely a branch of the wider field of stress biology (Van Straalen, 2003) offers the opportunity to unify approaches for mixture toxicity (multiple chemical) and multiple stressor (chemical/non-chemical) effect assessment.

Given the likely importance of exposures to multiple anthropogenic and natural stressors in the field, it is surprising that such combinations have not, to date, been a major priority for ecotoxicology (Calow and Forbes, 2003; Eggen et al., 2004; Højer et al., 2001; Holmstrup et al., 2000). To help address this knowledge gap, this study analyses the physiological and life-cycle response (short-term survival, reproduction) of a soil invertebrate species, the earthworm *Lumbricus rubellus*, to metal contaminated field soils under different temperature regimes in the laboratory and at different seasons in the field. Responses measured include survival and reproduction, metallothionein-2 (*MT-2*) expression as a biomarker of exposure, lysosomal membrane stability as a biomarker of cellular stress and earthworm tissue metal concentrations. The work was intended to establish firstly if exposure to temperatures that were below optimal, close to optimal and towards the upper thermal tolerance range of the species have any impact on earthworm propensity to intoxication and secondly, whether seasonal difference in environmental conditions (and other associated variable, such as food supply) result in physiological differences in worms at contaminated sites that affect biomarker responses.

2. Materials and methods

2.1. Collection of tests soils

Soils were collected in March 2002 from three sites under the deposition plume of a primary Cd, Pb and Zn smelter located at Avonmouth, South West England (Colgan et al., 2003). The sites were located at different distances (Site 1, 8.2 km; Site 2, 3.2 km; Site 3, 1.5 km) along a transect from the smelter (Filzek et al., 2004; Spurgeon et al., 2006) and have, thus, been subject to different levels of metal deposition over the 70 year lifetime of the plant (Coy, 1984). Site 1 was selected to typify the background metal levels for the region and thus, was intended to act as a field "control" against which measurements at the other two sites closer to the smelter could be compared. Measurements of metal concentration (nitric acid and 0.01 M $CaCl_2$ extractable) made indicated that this site cannot be seen a true negative control, although it was the sites at which lowest concentrations were found in the two different extracts for most metals (Table 1).

At each site, approximately 20 kg of soil was collected from the top 10 cm after removal of plant material, litter and fragmentation layers. The soil was mixed and then dried to constant weight at 60 °C. Aggregates were mechanically crushed (cleaning the equipment between samples), 2 mm sieved, mixed again and samples taken for analysis of pH, percentage loss on ignition (%LOI), and water holding capacity. Finally 1.5 L of the relevant soil was placed into each experimental container (plastic boxes with dimensions

Table 1
(a) mean ± SD of total (hot nitric acid extractable) metal concentrations (mg g^{-1} dry weight soil) and (b) mean ± SD of 0.01 M $CaCl_2$ extractable metal concentrations (mg g^{-1} dry weight soil) of six metals in soils collected from Sites 1, 2 and 3 and used in the laboratory bioassays with *L. rubellus* of six metals in soils collected from Sites 1, 2 and 3 and used in the laboratory bioassays with *L. rubellus*

(a)		As	Cd	Cu	Hg	Pb	Zn
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Site 1		12.5 ± 1.3 a	5.6 ± 0 a	27.1 ± 1.1 a	0.12 ± 0.02 a	106 ± 2 a	752 ± 59 a
Site 2		13.5 ± 2 a	24.2 ± 2.8 b	44.6 ± 5.3 a	0.16 ± 0.07 a	514 ± 53 c	2120 ± 23 b
Site 3		12.0 ± 2.6 a	29.9 ± 4.7 b	38.1 ± 9.7 a	0.15 ± 0.06 a	309 ± 77 b	3280 ± 44 c

(b)	Temp °C	As	Cd	Cu	Hg	Pb	Zn
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Site 1	10	0.016 ± 0.001	0.59 ± 0.01	<7 ± −	<0.25 ± −	<16 ± −	13.5 ± 3.1
	15	0.018 ± 0.004	1.68 ± 1.91	<7 ± −	<0.25 ± −	<16 ± −	11.9 ± 4.8
	20	0.079 ± 0.005	3.64 ± 2.04	<7 ± −	<0.25 ± −	<16 ± −	131 ± 2
Site 2	10	0.015 ± 0.001	2.07 ± 1.88	<7 ± −	<0.25 ± −	<16 ± −	15.1 ± 1.4
	15	0.017 ± 0.002	0.75 ± 0.21	<7 ± −	<0.25 ± −	<16 ± −	11 ± 4.6
	20	0.072 ± 0.017	2.49 ± 1.78	<7 ± −	<0.25 ± −	<16 ± −	111 ± 30
Site 3	10	0.017 ± 0.002	0.57 ± 0.04	<7 ± −	<0.25 ± −	<16 ± −	12.7 ± 3.4
	15	0.012 ± 0.004	0.80 ± 0.18	<7 ± −	< 0.25 ± −	<16 ± −	10.3 ± 3.4
	20	0.071 ± 0.002	2.54 ± 1.58	<7 ± −	<0.25 ± −	<16 ± −	103 ± 20