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A Comparative Study of the Effects of Metal Contamination on Collembola in the Field and in the Laboratory

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Abstract. We examined the species diversity and abundance of Collembola at 32 sampling points along a gradient of metal contamination in a rough grassland site (Wolverhampton, England), formerly used for the disposal of metal-rich smelting waste. Differences in the concentrations of Cd, Cu, Pb and Zn between the least and most contaminated part of the 35 metre transect were more than one order of magnitude. A gradient of Zn concentrations from 597 to 9080 μg g^{-1} dry soil was found. A comparison between field concentrations of the four metals and previous studies on their relative toxicities to Collembola, suggested that Zn is likely to be responsible for any ecotoxicological effects on springtails at this site. Euedaphic (soil dwelling) Collembola were extracted by placing soil cores into Tullgren funnels and epedaphic (surface dwelling) species were sampled using pitfall traps. There was no obvious relationship between the total abundance, or a range of commonly used diversity indices, and Zn levels in soils. However, individual species showed considerable differences in abundance. Metal "tolerant" (e.g., *Ceratophysella denticulata*) and metal "sensitive" (e.g., *Cryptopygus thermophilus*) species could be identified. Epedaphic species appeared to be influenced less by metal contamination than euedaphic species. This difference is probably due to the higher mobility and lower contact with the soil pore water of epedaphic springtails in comparison to euedaphic Collembola. In an experiment exposing the standard test springtail, *Folsomia candida*, to soils from all 32 sampling points, adult survival and reproduction showed small but significant negative relationships with total Zn concentrations. Nevertheless, juveniles were still produced from eggs laid by females in the most contaminated soils with 9080 μg g^{-1} Zn. *Folsomia candida* is much more sensitive to equivalent concentrations of Zn in the standard OECD soil. Thus, care should be taken in extrapolating the results of laboratory toxicity tests on metals in OECD soil to field soils, in which, the biological availability of contaminants is likely to be lower. Our studies have shown the importance of ecotoxicological effects at the species level. Although there may be no differences in overall abundance, sensitive species that are numerous in contaminated sites, and which may play important roles in decomposition ("keystone species") can be greatly reduced in numbers by pollution.

Keywords: Folsomia candida; Collembola; Zn; soil

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Introduction

Comparisons of the effects of metals in field soils on the soil fauna are hindered by between-site heterogeneity. Examples of this include, vegetation cover/complexity (Fratello et al., 1985; House et al., 1987; Wardle et al., 1993; Kampichler, 1999; Fountain and Hopkin, 2004), geographic location (Detis et al., 2000), soil type (Filser, 1991; Crommentuijn et al., 1997; Dunger and Wanner, 2001) and anthropogenic activity (Stork and Eggleton, 1992; Cancela da Fonseca and Sarkar, 1996; Lauga-Reyrel and Deconchat, 1999; Chernova and Kuznetsova, 2000). It is therefore difficult to deduce reliably whether between-site differences in species compositions are due to metal levels, or habitat differences. Ideally, if threshold values for the effects of metals on soil invertebrates are to be determined, ecological conditions should be as similar as possible to allow changes caused only by contaminants to be identified. It has also been suggested that comparisons need to be made within the same habitat along a concentration gradient (Belotti, 1998). The lack of a control (or ''clean'' soil), which is often a problem when sampling polluted areas, is also somewhat compensated for by using a gradient (Bruus Pedersen et al., 1999; Rusek and Marshall, 2000). Similarly, species missing on the more polluted part of the gradient could serve as indicators of unpolluted soil (Rusek and Marshall, 2000).

Collembolan indicators of soil *p*H have already been identified (Hågvar, 1984; Kopeszki, 1992; Nüss, 1994; Rusek and Marshall, 2000; Chagnon et al., 2001; Loranger et al., 2001). From a previous study in the Wolverhampton area, examining Collembola diversity from five sites (Fountain and Hopkin, 2004), it was found that species sensitive to the effects of metals were possible indicators of the pollution status of a site. It was also demonstrated that species diversity indices and total abundance of Collembola were uninformative as to the extent of contamination of a site. However, species indicative of high or low metal levels can give information on the effect that toxins are having on the Collembola community. Of the five sites previously studied in Wolverhampton, Ladymoor (a rough grassland site used for the disposal of metal-rich smelting waste) was considered the most contaminated with metals and was chosen for a gradient study.

Euedaphic (soil dwelling) and epedaphic (surface dwelling) species are affected in different ways by soil contamination (Bengtsson and Rungren, 1984; Moore et al., 1984; Kelly and Curry, 1985; Kuznetsova and Potapov, 1997). This may be because epedaphic species are usually more mobile and have less contact with the soil pore water than euedaphics (Hopkin, 1997). Edwards and Lofty (1974) found that euedaphic populations of Collembola were reduced more than epedaphics in soil treated with nitrogen. However, farming practices such as tillage only reduce numbers of epedaphic species (Moore et al., 1984). Hence, our study investigated the abundance and diversity of both surface and soil dwelling species to give a complete picture of the impact of metals on the Collembola. The soil was also analysed for its toxicity to the ''standard'' test springtail, *Folsomia candida*, following procedures outlined in the ISO laboratory test (Wiles and Krogh, 1998; ISO, 1999).

Materials and methods

Field Sampling

Ladymoor is a site within the Wolverhampton area (SO 944 952), which is part of the ''Black Country'' and has a long history of coal and iron ore mining and smelting. No control (''clean'') site could be found in this area because of the widespread industrial pollution. The site is a semi-natural open grassland, which was used historically for the dumping of material (slag) from the extraction of ''pig'' iron. A survey of the total and water soluble metal concentrations in the soil across the Ladymoor site was performed in Autumn 1999 to locate where the highest and lowest metal concentrations occurred. The metal distribution at the site was very heterogeneous, e.g., levels of Zn in soil ranged from background (122 $\mu g\ g^{-1}$) to nearly 10,000 $\mu g\ g^{-1}$ dry wt. over a distance of a few tens of metres. Therefore, this was considered an ideal site to study the effects of metals within a small area, of similar climate and vegetation type.

Soil cores, collembola extraction and pitfall traps

Soil cores were taken to a depth of 10 cm in accordance with Bengtsson and Rungren (1988) and Kaczmarek (1993). The cores were removed on 29th September 2000 and were 7 cm in diameter (total volume of each core = 390 cm^3). A 1 m^2 quadrat was placed at intervals of 5 m along a transect, 35 m in length. A core was taken at the corners of each quadrat to give a total of 32 soil samples. The cores (including surface vegetation) were placed into polythene bags for transport to the University of Reading laboratories on the same day and were put into individual Tullgren funnels. These were maintained until invertebrates had ceased emerging from the cores (approximately 7 days). The extracted fauna was stored in 70% alcohol.

Epedaphic species of springtail were sampled by means of pitfall traps (9 cm deep and 7 cm diameter), which were half filled with ethylene glycol, placed into the holes left by the soil cores and then removed after 1 week (6th October 2000). Springtails from the traps were stored in 70% alcohol.

Mounting, preparation and identification of Collembola

Collembola were sorted initially into families under a dissecting microscope (magnification x 140). A compound microscope (magnification x 1000) was employed for identifying to the species level. Individual Collembola were placed onto a cavity slide with 2 drops of distilled water and a coverslip was added. Some specimens required clearing with 10% potassium hydroxide to see their identifying structures (such as pseudocelli, ocelli or setae). Identification was carried out using the keys of Hopkin (2000) and Fjellberg (1998).

Soil analysis

Four sub-samples of soil were taken from each core for analysis after the soil fauna had been extracted. The soil was oven dried at 60 °C and then passed through a 1-mm aperture sieve to remove larger items of organic matter and stones. Organic matter content in the soils at Ladymoor was high (35–55%) (Fountain and Hopkin, 2004). Soil *p*H was recorded following the method of Spurgeon (1994). Water soluble and total metal content (Cd, Co, Cr, Cu, Fe, Pb, and Zn) were analysed by flame atomic absorption spectrophotometry (AAS-Varian Spectra-30 Flame with automatic background correction, see Hopkin, 1989 for further details).

For determination of water soluble metal, 1 g of soil from each replicate was weighed out into a 100-ml Pyrex flask. Double distilled water (50 ml) was added and the solution was allowed to stand overnight to let partial extraction begin. Subsequently, the flasks were shaken for 1 h on a Luckham R100 Rotatest shaker. This standard time of 1 h was used following Spurgeon (1994), as the shaking time effects the amount of metal desorbed. The solutions were left to settle out overnight and then 10 ml was decanted into a test tube ready for analysis.

The remaining sediment was oven dried at 60 °C, digested in boiling nitric acid and analysed (by AAS) to allow determination of the total metal content of the soil (see Hopkin, 1989; Fountain and Hopkin, 2001).

Folsomia candida exposure test

The survival and reproduction of the "standard" test springtail, *Folsomia candida*, was assessed in accordance with the ISO protocol (Wiles and Krogh, 1998; ISO, 1999). The original culture of *Folsomia candida* was from a single specimen donated by Dr. J. Wiles of Southampton University in 1994. Since then the Collembola have been maintained in our laboratory and have not been exposed to metals in that time.

The Collembola were maintained and cultured on a plaster of Paris: graphite powder substrate in clear plastic culture boxes at 20 ± 1 °C, a light: dark regime of 16:8 h, and a diet of dried active Baker's yeast *ad lib*.

The experimental procedure was performed as closely as possible to the protocol set out in the ISO (1999) report, which documents a standard test with *Folsomia candida* using an artificial soil (OECD, 2000) to test the effects of chemicals. The Ladymoor soils, from which the endemic soil fauna had been extracted, was oven dried at 60 °C for 24 h and then 30 g was weighed into plastic Sterilin pots (200 ml) with screw top lids. The soil was not sieved as this has been found to change its properties (e.g., increased nitrogen availability), which in turn can affect the experiment (Schlatte et al., 1998). The soil was frozen for 3 months at −20 °C (±2) (oven drying and freezing was used to prevent the survival of any remaining soil animals and their eggs that may interfere with the survival and reproduction of laboratory *Folsomia candida*). Subsequently, it was left to thaw at room temperature for one day after which distilled water (30 ml) was stirred into the soil. The lids were replaced and the pots were placed into a 20 °C controlled temperature room for 2 days to equilibrate. Four replicates from each of the 32 soil cores were prepared giving a total of 128 pots. After this time 2 mg of dried active Baker's yeast was added to provide the springtails (14 ± 1 days old) with an initial food source. Ten *Folsomia candida* were added to each pot using a fine moistened paintbrush, the lids were replaced and the pots maintained at 20° C (±1) for 28 days. The lids were removed twice a week to allow the exchange of air and the inside of the lids was sprayed lightly with distilled water to maintain 100% humidity.

At the end of the experiment (28 days) the soil was emptied into Tullgren funnels until all the *Folsomia candida* had been extracted (approx. 48 h). They were stored in 70% alcohol. The Tullgren method is unlike that of the ISO (1999) report, which uses flotation for extraction of springtails. Heat extraction was used instead, because of problems related to the high organic matter content of field soils. In such samples the organic matter floats to the top and obscures the Collembola making counting impossible. Adult and juvenile springtails were counted under a dissecting microscope.

Statistical analysis

Between-site and replicate comparisons were made using ANOVA and Fishers pairwise comparisons (Fishers individual error rate in Minitab 12.1 package). Regression analyses were used to examine the relationship between *Folsomia candida* (adults and juveniles) and metal concentrations. For the analysis of Collembola species diversity, the advice given by Southwood (1978), Magurran (1988) and Krebs (1999) was followed. No single species diversity index is superior for all circumstances, nor can it give a comprehensive picture of "richness" or "diversity" within or between samples (McAleece, 1997; Magurran, 1988; French and Lindley, 2000). For these reasons, a range of single figure diversity indices were used from two packages namely, Species Diversity and Richness II Package (Pisces Conservation Ltd, Lymington, England) and the BioDiversity Professional Beta (McAleece, 1997).

Indicator species were identified using a binary logistic regression (Minitab 12.1), which Smith and Anderson-Cook (2000) considered useful to assess the presence or absence of a species at a given metal concentration. The number of individuals present follows a binomial distribution, where the probability of presence is related to the metal concentration. Probability curves were plotted for indicator species.

Results

Field sampling

Concentrations of Co, Cr and Fe in Ladymoor soils were close to background levels as reported in Merian (1962) and are not shown. Levels of Cd, Cu, Pb and Zn were positively correlated in all soil samples (Pearsons correlation, $r > 0.601$). A comparison of the toxicities of these metals to *Folsomia candida*, and their relative concentrations in the soils suggests that Zn is the metal most likely to be responsible for any effects on Collembola at Ladymoor (Fountain and Hopkin, 2001; Hopkin and Spurgeon, 2001; Lock and Janssen, 2001a; Fountain and Hopkin, 2004). Thus, only the results for Zn will be shown throughout the rest of this paper (see Fountain and Hopkin, 2004, for details of other metals). Total and water soluble Zn concentrations were correlated and showed a positive exponential relationship (Pearsons correlation $r = 0.818$, Regression analysis $y = 4.4168e^{-0.0003}$, $R = 0.9256$, $P < 0.01$, Fig. 1). Water soluble Zn comprised less than 1.4% of the total Zn in all soil samples. It was expected that the four samples of soil taken from the corners of the quadrats could be treated as replicates at each of the eight sampling locations at 5 m intervals on the transect.

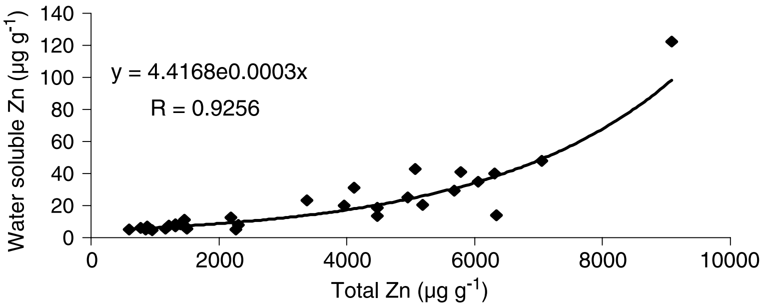

Figure 1. The exponential relationship between total and water soluble Zn in soil from Ladymoor (Pearsons correlation $r = 0.818$, Regression analysis $y = 4.4168e^{-0.0003}$, $R = 0.9256$, $P < 0.01$).

However, metal levels in the replicates were found to be significantly different from each other (ANOVA $F_{3,12} = 6.06–26.39$, Fishers pairwise comparisons $P < 0.01$), for both total and water soluble Zn. For this reason, the soil cores were not considered replicates for each of the eight sites and were treated as individual data points (Fig. 2).

Species data

Numbers of individual Collembola identified in this study were 367 and 796 in the soil cores and pitfall traps, respectively. Table 1 lists the 32 species found at Ladymoor on this particular sampling occasion. More species were exclusive to the soil cores (14 species) than the pitfall traps (four species) (Table 1).

No significant relationships were found between total or water soluble Zn concentrations in the soil cores/pitfall traps, and indices of Collembola diversity namely, abundance, Species Richness, Alpha index, Margalef index, Shannon–Weiner index, Simpson index or Shaneven (ANOVA $F_{1,30} = 0.00–4.05$, $P > 0.05$, data not shown).

However, certain species appear to be absent at high Zn concentrations, and others are more common. Collembola species abundance in the soil cores was plotted against total Zn concentration divided into three classes; 0–2000, 2000–5000 and 5000–10,000 µg g^{-1} (Fig. 3). The dominant species in the soil cores differed in the three Zn concentration classes. *Cryptopygus thermophilus* (CR THE) was dominant at 0–2000 µg Zn g^{-1}, *Isotomodes productus* (IT PRO) and *Isotoma notabilis* (IS NOT) at 2000–5000 µg Zn g^{-1} and *Folsomia fimetaria* (FO FIM) and *Isotomurus palustris* (IR PAL) at 5000–10,000 µg Zn g^{-1}. Some species are present exclusively at either high or low concentrations in soil cores (Figs. 4, 5). *Ceratophysella denticulata* (CE DEN) was more frequent at Zn

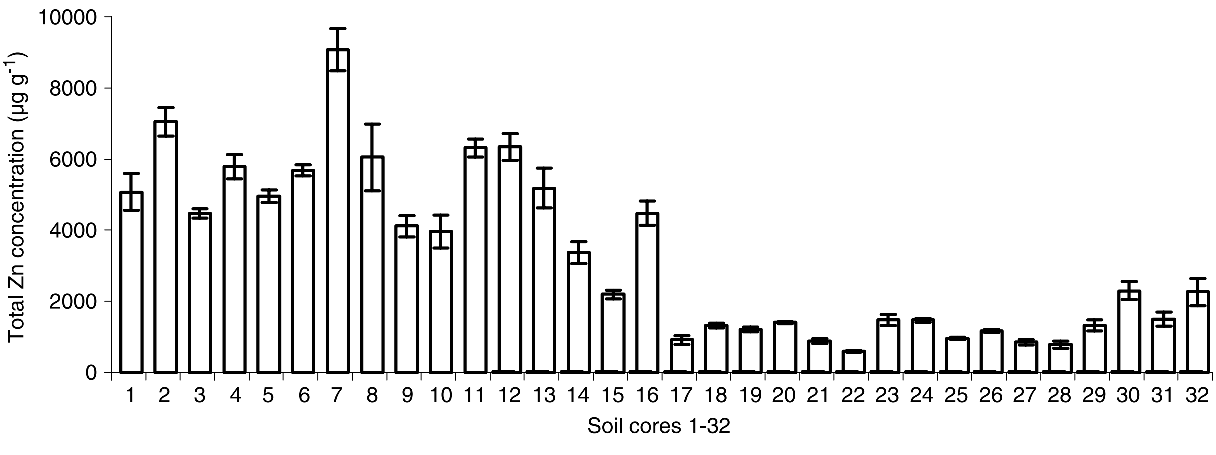

Figure 2. Histogram of mean total Zn concentration ($n = 4$, ± SE bars) in the 32 soil cores sampled from Ladymoor in the Autumn of 2000.

Table 1. Species of Collembola found at Ladymoor in soil cores and pitfall traps, sampled in the autumn of 2000

Species	Abbreviation	Soil core	Pitfall
Brachystomella parvula	BR PAR	✓	✓
Ceratophysella denticulata	CE DEN	✓	✓
Friesea truncata	FR TRU	✓	
Neanura muscorum	NN MUS	✓	✓
Protaphorura armata	PR ARM	✓	
Mesaphorura krausbaueri	MS KRA	✓	
Metaphorura affinis	MT AFF	✓	
Paratullbergia callipygos	PT CAL	✓	
Paratullbergia macdougalli	PT MAC	✓	
Stenaphorura denisi	SU DEN	✓	
Cyphoderus albinus	CY ALB	✓	
Entomobrya lanuginosus	EN LAN		✓
Lepidocyrtus lanuginosus	LE LAN	✓	✓
Orchesella villosa	OR VIL		✓
Cryptopygus thermophilus	CR THE	✓	✓
Folsomia candida	FO CAN	✓	
Folsomia fimetaria	FO FIM	✓	
Folsomia quadrioculata	FO QUA	✓	
Folsomia spinosa	FO SPI	✓	
Isotoma anglicana	IS ANG	✓	✓
Isotoma notabilis	IS NOT	✓	✓
Isotomodes productus	IT PRO	✓	
Isotoma tigrina	IS TIG		✓
Isotomurus palustris	IR PAL	✓	✓
Bourletiella arvalis	BO ARV	✓	✓
Deuterosminthurus pallipes	DE PAL	✓	✓
Deuterosminthurus sulphureus	DE SUL	✓	
Dicyrtomina minuta	DM MIN	✓	✓
Dicyrtomina ornata	DM ORN		✓
Sminthurinus elegans	SN ELE	✓	✓
Sphaeridia pumilis	SP PUM	✓	✓

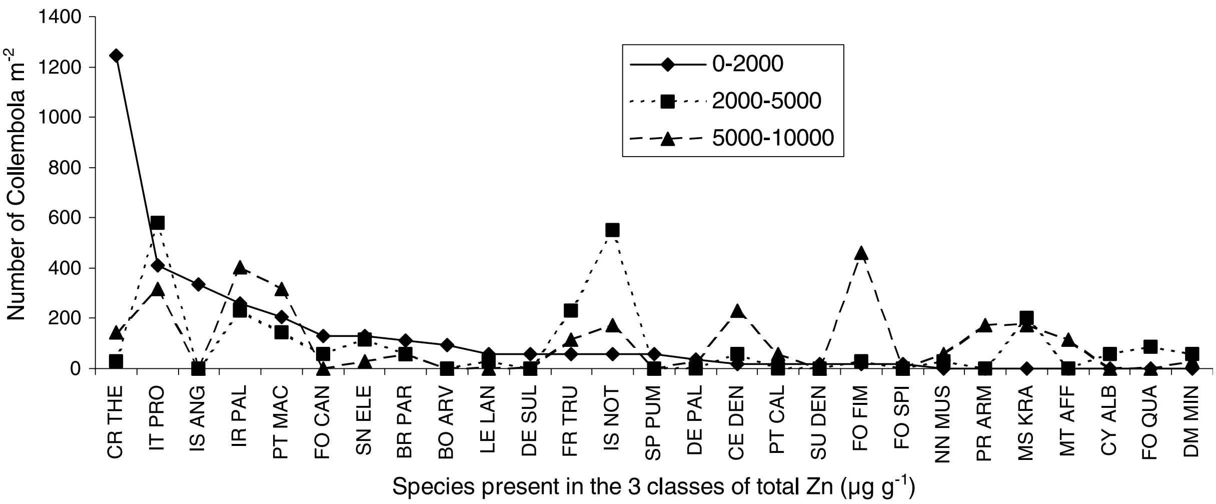

Figure 3. Ladymoor species abundance plots of Collembola in soil cores separated into three different classes of Zn concentrations (0–2000, 2000–5000 and 5000–10,000 µg g^{-1}), ranked in the order that species appear in the 0–2000 µg g^{-1} concentration. Full names of the species are given in Table 1.

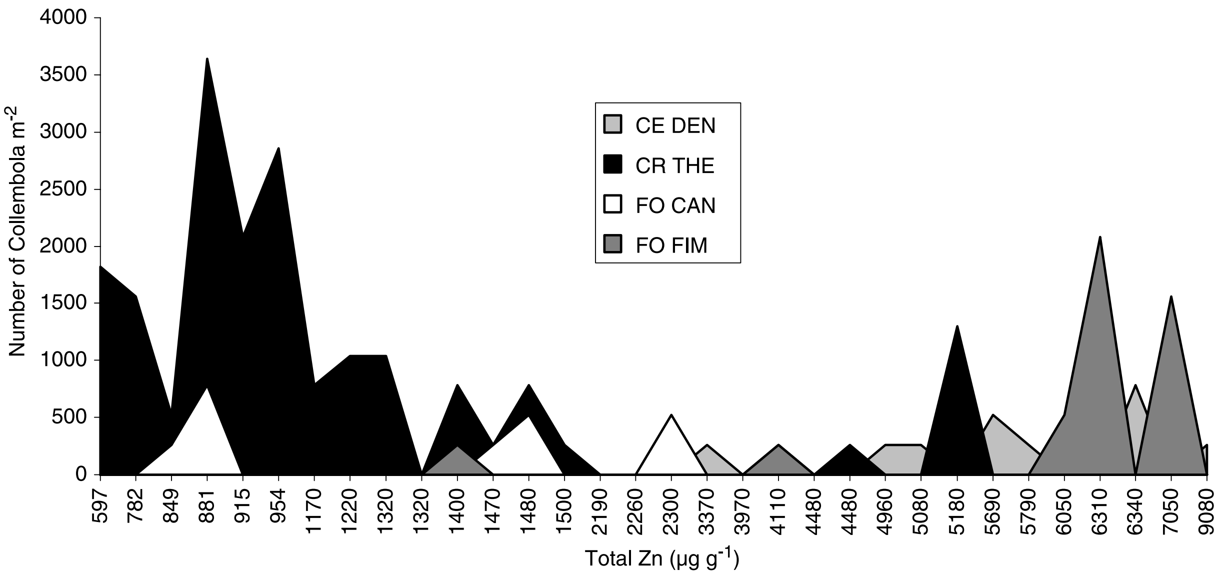

Figure 4. Relative abundance of *Ceratophysella denticulata* (CE DEN), *Cryptopygus thermophilus* (CR THE), *Folsomia candida* (FO CAN) and *Folsomia fimetaria* (FO FIM), from soil cores at Ladymoor. These species are possible indicator species, affected by metal contaminated soil.

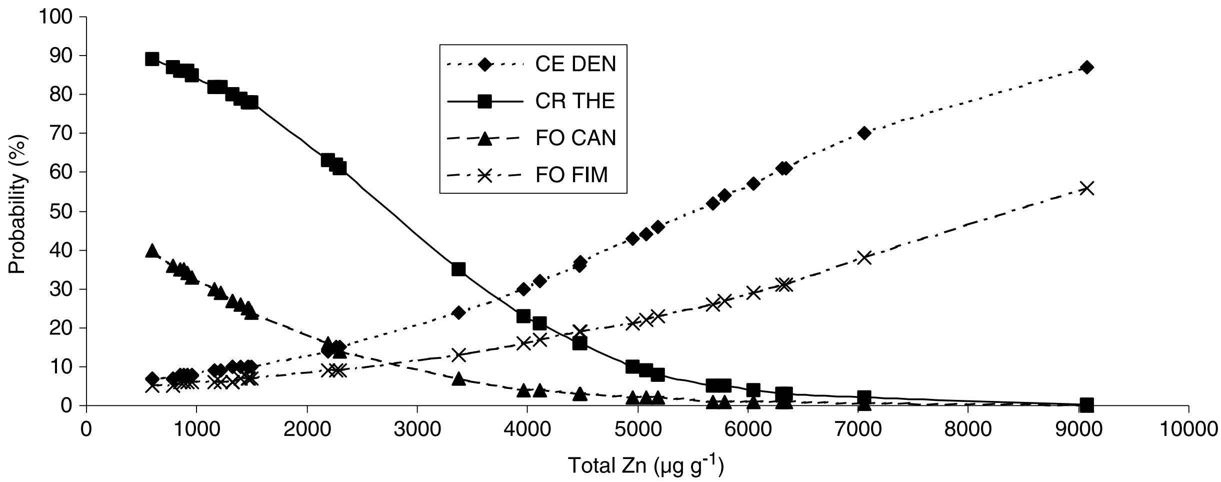

Figure 5. Probability plots for *Ceratophysella denticulata* (CE DEN), *Cryptopygus thermophilus* (CR THE), *Folsomia candida* (FO CAN) and *Folsomia fimetaria* (FO FIM), four possible Collembola indicator species from Ladymoor soil.

concentrations of more than 3380 µg g^{-1} (Binary logistic regression, $P < 0.01$). *Cryptopygus thermophilus* (CR THE) was found particularly at Zn concentrations below 4960 µg g^{-1} and *Folsomia candida* (FO CAN) at concentrations below 2300 µg g^{-1} (Binary logistic regression, $P < 0.05$). *Folsomia fimetaria* (FO FIM) did not have a statistically significant higher prevalence at high Zn concentrations, although the small number of individuals found at 1500 µg g^{-1} may influence this result. Results for water soluble Zn concentrations and indicator species were in agreement with those of total Zn in soil cores.

The population densities of some Collembola species in pitfall traps were also related to Zn concentrations (Figs. 6, 7). As with the soil cores, *Ceratophysella denticulata* (CE DEN) was more common at high Zn concentrations (Binary logistic

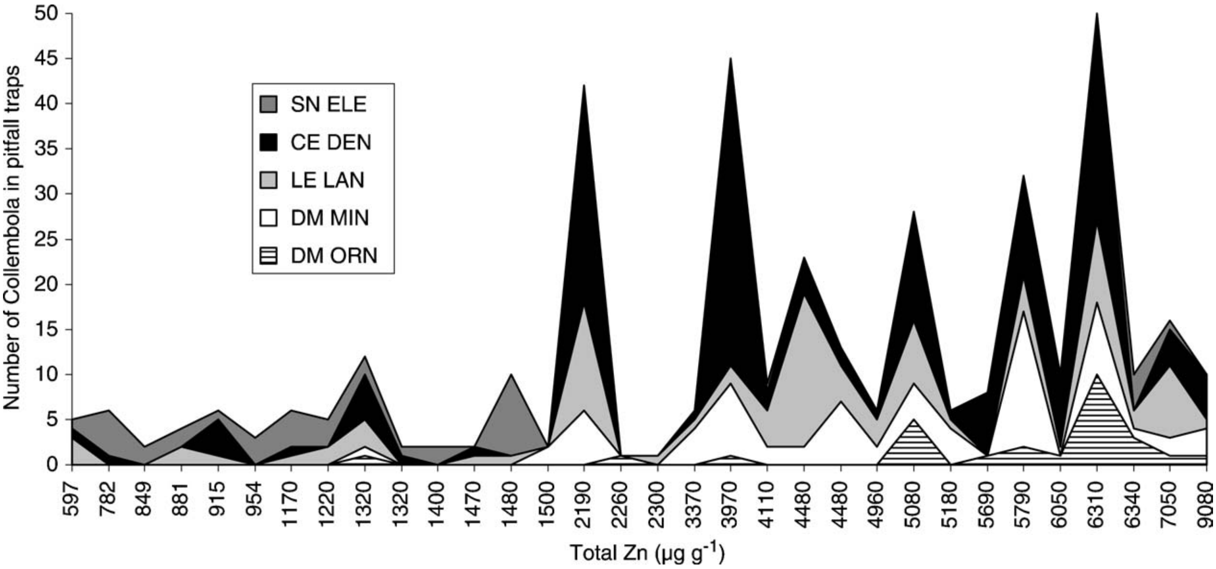

Figure 6. Relative abundance of *Ceratophysella denticulata* (CE DEN), *Lepidocyrtus lanuginosus* (LE LAN), *Dicyrtomina minuta* (DM MIN), *Sminthurinus elegans* (SN ELE) and *Dicyrtomina ornata* (DM ORN), from pitfall traps at Ladymoor. These species are possible indicator species, affected by metal contaminated soil.

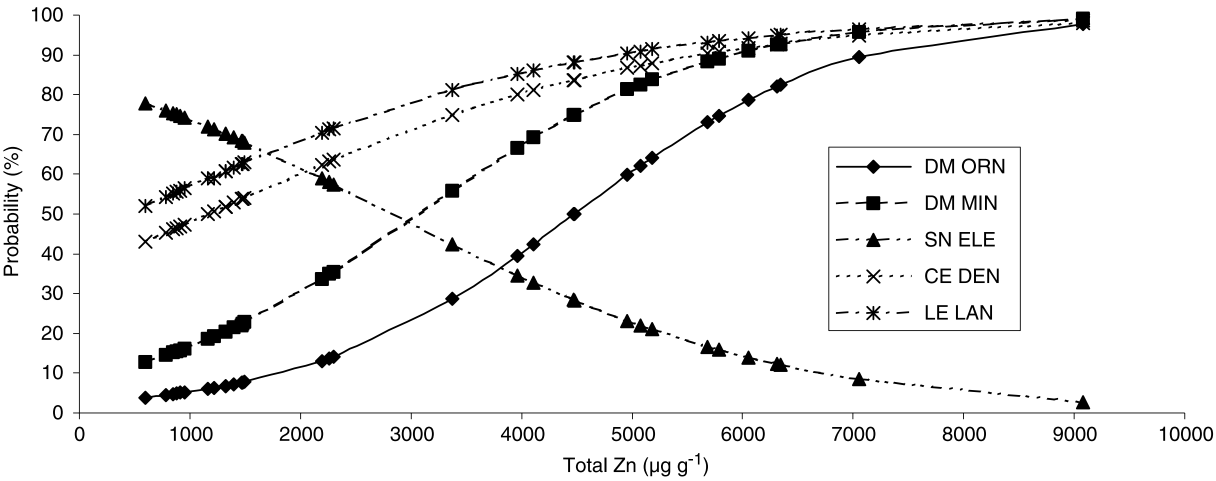

Figure 7. Probability plots for *Ceratophysella denticulata* (CE DEN), *Lepidocyrtus lanuginosus* (LE LAN), *Dicyrtomina minuta* (DM MIN), *Sminthurinus elegans* (SN ELE) and *Dicyrtomina ornata* (DM ORN) from pitfall traps at Ladymoor.

regression, $P < 0.05$). *Lepidocyrtus lanuginosus* (LE LAN), *Dicyrtomina ornata* (DM ORN) and *Dicyrtomina minuta* (DM MIN) were also more prevalent at high Zn concentrations (Binary logistic regression, $P < 0.05$). *Sminthurinus elegans* (SN ELE) was found to occur with less frequency at high Zn concentrations (Binary logistic regression, $P < 0.01$). These results were in agreement for both total and water soluble Zn.

Folsomia candida exposure test

The number of juveniles produced was positively related to the number of adults that survived in the soil ($y = 95.637e^{-51.978}$, $R = 0.8361$, ANOVA $F_{1,30} = 69.71$, $P < 0.01$). The number of adults was significantly negatively correlated with the total (Fig. 8) and water soluble Zn concentrations ($y = -0.0003e^{6.3751}$, $R = 0.4271$, ANOVA $F_{1,30} =$

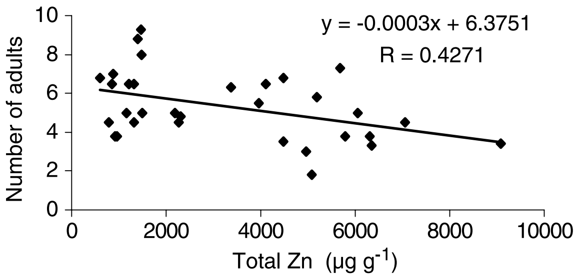

Figure 8. Regression analysis of adult survival at the end of a 4 week exposure test to Ladymoor soil, compared to total Zn concentrations ($y = -0.0003e^{-6.3751}$, $R = 0.4271$, ANOVA $F_{1,30} = 6.69$, $P < 0.05$).

6.69, $P < 0.05$; $y = -0.0277e^{5.8895}$, $R = 0.3615$, ANOVA $F_{1,30} = 4.51$, $P < 0.05$, respectively). The number of juveniles was negatively correlated with total Zn concentrations ($y = -0.0345e^{-674.61}$, $R = 0.4033$, ANOVA $F_{1,30} = 5.83$, $P < 0.05$, Fig. 9), but less so with water soluble Zn concentrations ($y = -3.0182e^{622.56}$, $R = 0.3438$, ANOVA $F_{1,30} = 4.02$, $P = 0.054$, plot not shown).

Discussion

At Ladymoor, the location of the transect was chosen to minimise variability due to factors other than metal concentrations in the soil, as suggested by Filser (1991). The ecological conditions were comparable and the survey was conducted along a gradient (Belotti, 1998; Bruus Pedersen et al., 1999; Rusek and Marshall, 2000).

A high variability in Zn concentrations was found between soil samples only one metre apart. For this reason, the original eight sites along the transect were not considered as replicates. Instead,

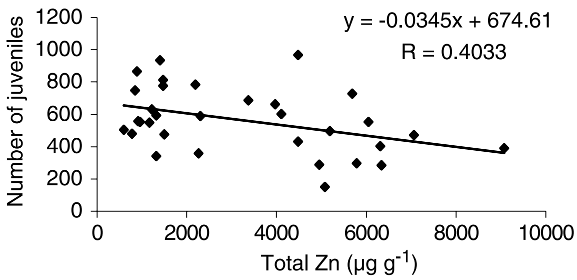

Figure 9. Regression analysis of juvenile production at the end of a 4 week exposure test to Ladymoor soil, compared to total Zn concentrations ($y = -0.0345e^{-674.61}$, $R = 0.4033$, ANOVA $F_{1,30} = 5.83$, $P < 0.05$).

each sample/core was treated as an individual site. Similarly, Van Gestel et al. (2001), found that soil heterogeneity hindered replication on a site contaminated with oil. Soil heterogeneity is important for the survival of species at contaminated sites (Filser et al., 2000; Niklasson et al., 2000) and Sjögren (1997) showed that Collembola were able to disperse in soil and tended to settle in unpolluted areas when introduced into boxes containing metal contaminated and "clean" soil. Total concentrations of Zn at Ladymoor ranged from 597 µg g^{-1} in the cleanest soil (core 22) to 9080 µg g^{-1} in the most contaminated (core 7, Fig. 2). The exponential relationship between total and water soluble Zn (Fig. 1) occurs because soils generally have an adsorption maximum: when a site becomes saturated with metals, the amount of the free ion form becomes higher due to the lower buffering capacity of the soil (Alloway, 1990). This effect is seen particularly in soil from core 7.

Collembola abundance as an estimate of toxicity has been useful with studies using pesticides (e.g., Fox, 1967; Sanocka-Woloszyn and Woloszyn, 1970; Ishibashi et al., 1978; Chakravorty and Joy, 1990; Filser, 1995; Bishop et al., 1998; Peveling et al., 1999; Holland et al., 2000), atmospheric pollution (Moursi, 1962; Bressan and Paoletti, 1997), PCPs (Salminen and Haimi, 1996, 1997 and 1998), and metal aerial deposition or metal "spiked" soils (Strojan, 1978; Haimi and Siirapietikainen, 1996; Smit and Van Gestel, 1996; Bruus Pedersen and Van Gestel, 2001). However, total Collembola abundance does not seem to be affected in long-term (decades) metal contaminated sites (this study; Bengtsson and Rundgren, 1988; Smit and Van Gestel, 1996; Russell and Alberti, 1998; Bruce et al., 1997 and 1999; Bruus Pedersen et al., 2000; Murray et al., 2000). Abundance may not change because Collembola are relatively resistant to metals due to their ability to excrete contaminants stored in the gut lining when they moult (Humbert, 1974; Joosse and Buker, 1979; Kronshage, 1992; Pawert et al., 1996). More significant than the estimation of abundance is the effect that both pesticides and metals have on species composition. Applications of metal contaminated sewage sludge to soil may increase the number of Collembola, but it also alters the proportions of individual species (Lübben, 1989). Species that are prevalent in uncontaminated soils may be better competitors, but less able to tolerate metals. Therefore, when these populations are reduced, more tolerant and competitively suppressed species can increase in number. Russell and Alberti (1998) found that the numbers of euedaphic species at a clean site were higher than those at a metal contaminated site and there were more hemiedaphic and epedaphic species in the contaminated site.

Species diversity indices were not consistently related to the concentration of metals in the soil. The actual species present appears to be a more reliable indicator of metal pollution. Filser et al. (1995) has suggested using Collembola species abundance as an indicator of metal pollution, because even though they may have decreased sensitivity compared to some other soil invertebrates, they show distinct differences in sensitivity between species. At Ladymoor, *Cryptopygus thermophilus* and *Folsomia candida* seem to be metal sensitive (Figs. 4, 5). Lübben (1989) also found *Cryptopygus thermophilus* to be metal sensitive. *Ceratophysella denticulata* is metal tolerant and this agrees with findings by Cole et al. (2001). *Folsomia fimetaria* is not a statistically significant indicator species, but is certainly more prevalent at high metal concentrations. This has obvious implications for its use as a "standard" test springtail in ecotoxicological tests with metals. *Folsomia candida* is metal sensitive and therefore more likely to be an environmentally protective test species, at least where metal contamination is concerned.

Pitfall trapping has been demonstrated to be a reliable method for sampling epedaphic springtails (Berbiers et al., 1989), although up to 18 factors have been identified which may influence the "catch" of soil animals (Adis, 1979). These varied from climate and vegetation cover (Melbourne, 1999), to the dimensions and placement of the traps themselves. The use of soil cores and pitfall traps certainly yielded more species of Collembola (this study) than in studies using soil cores alone (Fountain and Hopkin, 2004) and can increase the number identified by 17% (Therrien,1999). With regard to epedaphic soil animals, Bengtsson and Rungren (1984) found beetles and spiders to be unreliable indicators of metal pollution due to their high mobility. In this study, certain species of Collembola seemed to be affected by metal concentrations (Figs. 6, 7).

Ceratophysella denticulata was more prevalent at high Zn concentrations; however, the probability of its occurrence on the surface at lower concentrations of Zn was over 40%, compared to below 10% in the soil. This may be because they are less exposed to metals whilst on the soil surface. The same is true for *Lepidocyrtus lanuginosus*; although it was frequent at high metal concentrations, it was also likely to be found more than 40% of the time at low Zn concentrations (597 $\mu g\ g^{-1}$).

Two other epedaphic species, also found with high frequency at elevated metal concentrations were, *Dicyrtomina ornata* and *Dicyrtomina minuta*. No literature was found relating to metal tolerance in these species. They are considered cosmopolitan and can be found prevailing in habitats not contaminated by metals. *Sminthurinus elegans* is common at low Zn levels with a less than 5% probability of being present at 9080 $\mu g\ g^{-1}$, possibly a more reliable indicator of uncontaminated soil.

Epedaphic species are generally more mobile and in less contact with soil pore water than soil species, resulting in different effects of chemicals. The pesticide, methiocarb, had a significant effect on populations of Onychiuridae (soil dwelling species) by reducing numbers, whilst the epedaphic Collembola were not affected (Kelly and Curry, 1985). Conversely, epedaphic Collembola were reduced by tillage (Moore et al., 1984). Both euedaphic and epedaphic species were affected by metals in soil in this study, but it could be assumed from evidence of the presence of *Ceratophysella denticulata* that epedaphic individuals are less at risk through metal contamination than euedaphic species.

Species such as *Folsomia fimetarioides* (Bengtsson and Rungren, 1988; Tranvik and Eijsackers, 1989; Haimi and Siirapietitkainen, 1996) and *Isotoma olivacea* (Hågvar and Abrahamsen, 1990) have been suggested as indicators of high metal contamination. These species were not present in this study, so comparisons cannot be made.

Indicator species probably arise as a result of lowered competition from more common species that have experienced population reductions as a result of the contamination. In a series of experiments using acidification of soil, Hågvar (1984) has suggested that competition may play a role in changing communities under stressed conditions. In the same study, it was determined that the number of predatory mites did not have a significant effect on the Collembola. Competition seems to be one of the most important factors (Rusek and Marshall, 2000) that affect the collembolan community.

There was a positive correlation between the number of adults and the number of juveniles of *Folsomia candida* exposed to the field soils from Ladymoor. Adult survivorship and reproduction was reduced at high concentrations of Zn. Although we were unable to deduce at which life stage the collembolans were affected within this experiment (i.e., egg production/hatchability, juvenile survival or adult mortality early in the test), the overall effect is a reduction in the population, which is detrimental to the survival of a species at a contaminated site.

Ge et al. (2000) found that metals in urban soils were mainly in their stable forms and that bioavailability was low, as seems to be the case at Ladymoor. Smit and Van Gestel (1996) found no effect on the growth or reproduction of *Folsomia candida* with water soluble Zn concentrations of 2.5 $\mu g\ g^{-1}$ (4 weeks). Water soluble Zn concentrations at Ladymoor reached over 120 $\mu g\ g^{-1}$ and were not quite high enough to reduce Collembola populations significantly ($y = -3.0182e^{622.56}$, $R = 0.3438$, ANOVA $F_{1,30} = 4.02$, $P = 0.054$).

Differences in toxicity between freshly contaminated and aged soils suggest that the use of water soluble metal concentrations are a more reliable prediction of the effects of metals on springtails (Smit et al., 1997; Smit and Van Gestel, 1998). Often the toxicity of metals in the standard OECD soil is higher than in field soils (Vijver et al., 2001; Lock and Janssen, 2001b). Lock and Janssen (2001a) and Fairbrother et al. (1999) have expressed the need to use aged or natural soils representative of the area under consideration, as being more indicative of field conditions. In this study, the results for total metal were more significant than those for water soluble Zn. This was not expected, as it is thought that the water soluble fraction of the metal is that which would be affecting Collembola.

Crommentuijn et al. (1997) demonstrated that the bioavailability of Cd was not determined by the water soluble concentrations alone and suggested that soil characteristics may also play a role. The relationship between *F. candida* and metal uptake via pore water was less obvious than for oligochaetes in a study by Vijver et al. (2001) suggesting that the uptake mechanisms of metals are not purely from pore water. These mechanisms (uptake via the epidermis, ventral tube and gut, via food) were discussed by Fountain and Hopkin (2001).

Loss of biodiversity is said to detrimentally alter an ecosystem (Naeem et al., 1994). Functional diversity of an ecosystem is more important than species diversity for the performance of that system (Bengtsson, 1998). Methods for measuring this need to be explored further, as it has been demonstrated in this study that the number and abundance of species may not necessarily change at a polluted site. Species composition of Collembola has an effect on nitrogen turnover (Mebes and Filser, 1998) and whilst the number of soil animals may increase (as seen with some pesticide regimes), the diversity and biomass may decrease (Menhinick, 1962). The composition and therefore, how the species functions in an ecosystem may change with pollution and the new dominant species might no longer fulfil the former role in the ecosystem.

Problems also arise with field soils where there may be unseen effects of mixed toxicity of metals in the water soluble phase (Nottrot, 1987). Van Gestel and Hensbergen (1997) found that Zn in soil increased the presence of Cd in the water soluble portion, although the reverse was not true for the effect of Cd on Zn.

Different species indicators will be required for different toxins, e.g., metals, herbicides, insecticides, fungicides, crude oil etc., and this may need to be narrowed to the actual chemical (e.g. for insecticides; cypermethrin, DDT, pirimicarb, etc.) and soil type.

Conclusions

Soil heterogeneity hinders ecotoxicological testing due to difficulties with replication, which impedes reliable statistics or analysis. However, pockets of relatively clean soil may act as refuges within a contaminated site sustaining soil invertebrate biodiversity. In Ladymoor soils the proportion of Zn which was water soluble was always less than 1.0%, except at the highest level (9080 $\mu g\ g^{-1}$) where the value was 1.4%. This finding is consistent with the cation exchange sites becoming saturated, a well-known phenomenon in soil biology (Alloway, 1990). Total abundance of Collembola is not a reliable estimate of the effects of soil contamination and species diversity indices are not recommended for the interpretation of metal contaminated sites. Species composition is a more useful tool for assessing the impact of pollutants. Metal tolerant species are a good indicator of poor soil "health". However, caution must be taken in the interpretation of such data, as some of these species may also be found in abundance at "clean" sites. Metal-sensitive species absent from metal contaminated soils may be a better tool for assessing soil pollution effects. Species present at the soil surface appear to be influenced less by metal contamination than those living within the soil. This is probably due to their high mobility and relatively low contact with the soil pore water. The latter will depend on the application and type of toxin that contaminates the soil.

In soils from the most contaminated cores at Ladymoor, *Folsomia candida* populations were significantly reduced in comparison with the least contaminated soil cores. Hence, soil tests using *Folsomia candida* to assess the "health" of soil are recommended as a standard procedure for testing contaminated field soils.

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