



## Biodiversity of Collembola in Urban Soils and the Use of *Folsomia candida* to Assess Soil ‘Quality’

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**Abstract.** The effects of metal contamination on natural populations of Collembola in soils from five sites in the Wolverhampton area (West Midlands, England) were examined. Analysis revealed that metal concentrations were elevated above background levels at all sites. One location in particular (Ladymoor, a former smelting site) was highly contaminated with Cd, Cu, Pb and Zn at more than 20 times background levels. Biodiversity indices (Shannon–Weiner, Simpson index, Margalef index, alpha index, species richness, Shaneven (evenness) and Berger–Parker dominance) were calculated. Of these indices, estimates of species richness and evenness were most effective at highlighting the differences between the Collembola communities. Indeed, the highest number of species were found at the most contaminated site, although the Collembola population also had a comparatively low evenness value, with just two species dominating. The number of individuals per species were allocated into geometric classes and plotted against the cumulative number of species as a percentage. At Ladymoor, there were more geometric classes, and the slope of the line was shallower than at the other four sites. This characteristic is a feature of polluted sites, where a few species are dominant and most species are rare. The Ladymoor soil also had a dominance of *Isotomurus palustris*, and was the only site in which *Ceratophysella denticulata* was found. Previous studies have shown that these two species are often found in sites subject to high metal contamination. Survival and reproduction of the “standard” test springtail, *Folsomia candida* (Willem), were determined in a 4 week exposure test to soils from all five sites. Mortality was significantly increased in adults and reproduction significantly lower in the Ladymoor soil in comparison to the other four sites. This study has shown that severe metal contamination can be related to the population structure of Collembola in the field, and performance of *F. candida* (in soils from such sites) in the laboratory.

**Keywords:** Collembola; metal; Cd, Cu, Pb, Zn; *Folsomia candida*; diversity

### Introduction

Collembola (springtails) are abundant and widespread in soil ecosystems and are important members of the decomposer community (for review see Hopkin, 1997). The effects of toxins on

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Collembola in field conditions have been reasonably well studied in relation to pesticides and metals. Several researchers have shown that pollution of soil by a wide range of contaminants can change the species composition within the collembolan community in comparison to "clean" sites (e.g. Abel and Larink, 1994; Nüss, 1994; Chernova et al., 1995; Filser et al., 1995; Moldenke and Thies, 1996; Salminen and Haimi, 1996; Frampton, 1997; Kuznetsova and Potapov, 1997; Chernova and Kuznetsova, 2000; Rebecchi et al., 2000; Cole et al., 2001). Anthropogenic activities may have persistent and long lasting effects on Collembola (Frampton, 2001), although with long-term (centuries) exposure, springtails can become tolerant to metals (Hågvar and Abrahamsen, 1990; Posthuma and Van Straalen, 1993). Soil microarthropods may have a high degree of site-specificity and are potentially good bioindicators of pollution (Van Straalen, 1997). Steiner (1995) found that Collembola species richness decreased with increasing pollution and Naem et al. (1994) reported that a declining biodiversity is consistent with reduced ecosystem function. However, functional diversity can be difficult to measure and so species diversity is usually estimated instead (Bengtsson, 1998).

Numerous researchers have conducted laboratory tests using "standard" organisms to assess the toxicity of pollutants in standard soils (Løkke and Van Gestel, 1998). Other authors have brought contaminated soils from the field into the laboratory to assess effects on survival and reproduction of such organisms. One of the most widely used species is the "standard" test springtail, *Folsomia*

*candida* (Willem). The use of *in situ* soils is more representative of field conditions in a particular area than many of the artificial soils, e.g. OECD standard soil (Fairbrother et al., 1999; OECD, 2000). Such experiments are useful to test the toxicity of soils at a site or for the assessment of soils before and after remediation (Achazi et al., 1997; Kratz and Riesbeck, 1998; Haimi, 2000; Fava et al., 2000; Juvonen et al., 2000; Van Gestel et al., 2001). The procedure for the standard ISO laboratory test can be followed (ISO, 1999; Wiles and Krogh, 1998), but replacing OECD soil with field soil.

In this paper, a survey of the effects of metal pollution on the biodiversity of Collembola at five urban sites in the Wolverhampton area has been carried out. In addition, the performance of *F. candida* in soils from the five sites was observed in order to assess the potential of Collembola as indicators of ecosystem change in response to contamination.

## Materials and methods

### Field sampling

Field sampling was carried out at five sites in Wolverhampton, West Midlands, England (Table 1) namely, East Park Grassland (EPG), East Park Woodland (EPW), Peascroft Park Woodland (PPW), Bilston Gas Works (BGW) and Ladymoor (L). Wolverhampton is part of the "Black Country" and has a long history of coal and iron ore mining and smelting. Hence, no control (clean) site could be found within the

Table 1. Description of the five Wolverhampton sites from which soil cores were removed, including their abbreviations and OS reference

Site	Abbreviation	OS reference	Site description
East Park Grassland	EPG	SO 933 980	Mown grassland playing field, capped with 2.5 cm of clay and approx. 15 cm of topsoil
East Park Woodland	EPW	SO 935 977	Mixed, unmanaged woodland planted on spoil heaps, surface layer of leaf litter (3 cm depth)
Peascroft Park Woodland	PPW	SO 951 970	Predominantly an unmanaged, ash woodland with minimal levelling and capping, originally used for coal and iron ore mining, surface layer of leaf litter (3 cm depth)
Bilston Gas Works	BGW	SO 936 966	Disused gas works, rough grassland
Ladymoor	L	SO 944 952	Semi-natural open grassland, which was used for the dumping of material (slag) from the extraction of 'pig' iron

Wolverhampton area because of this widespread industrial pollution.

#### *Soil cores and Collembola extraction*

More than 90% of Collembola inhabit the top 10 cm of soil (Bengtsson and Rungren, 1988; Kaczmarek, 1993). Thus, soil cores of 10 cm depth were considered to be sufficient to sample most of the springtails. Cores of 16 cm diameter were removed on 13th April, 6th July and 5th October 1999. Four replicates 1 m apart in a quadrat were extracted from each site. The cores, including the surface vegetation, were placed into polythene bags for transport to the University of Reading laboratories where they were put into Tullgren funnels (the organisms extracted by placing the core on a 5 mm mesh, with a source of heat above). These were maintained until no more organisms emerged from the cores (ca. 1 week). Subsequently the fauna were sorted and stored in 70% alcohol.

#### *Mounting, preparation and identification of Collembola*

Collembola were sorted initially into Families under a dissecting microscope (magnification 140×). A compound microscope (magnification 1000×) was employed for identifying to 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*

Sub-samples of soil were taken from the cores 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 (OM) and stones. For the OM and metal profiles, samples were taken from the sides of the hole left after the removal of each soil core (at 0–2, 2–4, 4–6, 6–8 and 8–10 cm).

Soil pH was measured by adding 10 ml of distilled water to 10 g of soil. Samples were shaken thoroughly for 1 min and then left to settle. The

pH meter (Hanna HI 931410) was calibrated before use and then the electrode positioned in the supernatant. The electrode was then gently shaken and a reading recorded after 1 min, rinsing in-between samples with distilled water.

The protocol for measuring water soluble metal was as follows: double distilled water (50 ml) was added to 1 g of soil and the solution was allowed to stand overnight to let partial extraction begin. Subsequently, the flasks were shaken for 1 hour on a Luckham R100 Rotatest shaker (100 rpm). This standard time of 1 h was used as the shaking time effects the amount of metals 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 to determine the total metal content of the soil (see Hopkin, 1989; Fountain and Hopkin, 2001).

Water soluble and total metal content (Cd, Co, Cr, Cu, Fe, Ni, Pb, and Zn) were analysed by flame atomic absorption spectrophotometry (Varian Spectra – 30 Flame with automatic background correction). The protocol was validated by analysis of a standard reference soil (calcareous loam from the Community Bureau of Reference, Brussels), as recommended by Hopkin (1989). Measured values were within 10% of certified values.

#### *Folsomia candida exposure test*

The survival and reproduction of *F. candida* was studied in the soils from the five sites (collected in October 1999). The original culture of *F. candida* was donated by Dr. J. Wiles of Southampton University in 1994. Since then the Collembola have been maintained in our laboratory at the University of Reading and have not been exposed to metals in that time.

Collembola were maintained and cultured according to ISO (1999) and Wiles and Krogh (1998) on a plaster of Paris:graphite powder substrate in clear plastic culture boxes at a temperature of 20 ± 1 °C, a light:dark regime of 16:8 h and fed dried active Baker's yeast *ad lib*.

The ISO (1999) protocol was followed substituting the artificial standard soil (OECD, 2000), with field soils from the five sites. Soil 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 200 ml plastic Sterilin pots with screw top lids, after which it was frozen for 3 months at  $-20\text{ }^{\circ}\text{C}$  ( $\pm 2$ ). Oven drying and freezing prevents the survival of any remaining soil animals and their eggs that may interfere with the survival and reproduction of laboratory *F. candida*. The soil was not sieved as this has been found to change its properties (e.g. increased nitrogen availability), which in turn can affect experiments (Schlatte et al., 1998). After thawing at room temperature for one day, distilled water (30 ml) was stirred into the soil. The lids were replaced (4 replicates per soil core per site,  $n = 16$  replicates per site,  $n = 80$  pots) and the pots were maintained at  $20\text{ }^{\circ}\text{C}$  for 2 days to equilibrate. After this time 2 mg of dried active Baker's yeast was added to provide the springtails with an initial food source. Ten *F. candida* ( $14 \pm 1$  days old) were added to each pot using a fine moistened paintbrush, the lids were replaced and the pots maintained at  $20\text{ }^{\circ}\text{C}$  ( $\pm 1$ ) for 28 days. Twice a week the lids were removed to allow the exchange of air and the inside of the lids sprayed lightly with distilled water to maintain the humidity.

At the end of the experiment (28 days) the soil was emptied into Tullgren funnels and the *F. candida* were extracted into tubes of 70% alcohol. This method was used instead of flotation (ISO, 1999) because the OM in the field soils floated to the top and obscured the Collembola, making counting impossible. Adult and juvenile Collembola were counted under a dissecting microscope.

#### Statistical Analysis

In contaminated field sites metals do not usually occur at the same concentrations. The relative toxicity factor ( $T_F$ ) for the metals at each of the Wolverhampton sites was calculated according to the method of Hopkin and Spurgeon (2001) and using the relative toxicities of metals in contaminated food to *F. candida* (the ratios are similar in soil and food, see Fountain and Hopkin, 2001). This is achieved by calculating the concentration of each metal relative to one of the metals, e.g. Cd ( $C_{Cd}$ ) and the toxicity of each metal related to the same metal ( $T_{Cd}$ , e.g. EC50s). The  $T_F$  is then estimated by dividing  $C_{Cd}$  by  $T_{Cd}$ . The metal with the highest  $T_F$  value is the one most likely to be

causing a problem in the soil. This enables the identification of the metal within a "cocktail", which is most likely to be causing deleterious effects in the field (see Fountain and Hopkin, 2001 for further details). In this study Cu was used to calculate  $T_F$  since Cd was sometimes below the detection limit of the analytical methods used.

Between-site and sample comparisons were made using ANOVA and Fishers pairwise comparisons (Fishers individual error rate in Minitab 12.1 package). For the analysis of Collembola species diversity, the advice given by Southwood (1978), Magurran (1988) and Krebs (1999) was followed. No one species diversity index could be said to be superior for all circumstances, or give a comprehensive picture of "richness" or "diversity" within or between samples (McAleece, 1997; Magurran, 1988 and 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 BioDiversity Professional Beta (McAleece, 1997).

Rank abundance graphs (or species abundance curves (log abundance on species rank), Whittaker plots in Krebs, 1999) were analysed in the Species Diversity and Richness II Package to test the species distribution. The test gives the observed and expected species abundance and uses a  $\chi^2$  to examine which model the curve fits.

## Results

#### Field sampling

When comparing concentrations of metals in the soils (Cd:Cu:Pb:Zn) at each site in relation to their toxicities to *F. candida* in the laboratory (relative to Cu, from Fountain and Hopkin, 2001) the  $T_F$  (toxicity factor) for each site was calculated as  $<0.1:1.0:0.1:0.1$  for EPG,  $<0.1:1.0:0.1:0.2$  for EPW,  $<0.1:1.0:0.2:0.8$  for Peascroft,  $<0.1:1.0:0.1:1.5$  for Ladymoor and  $<0.1:1.0:0.2:1.0$  for BGW. Hence, at Ladymoor (the most contaminated site), Zn (with the highest  $T_F$  of 1.5) is the metal most likely to be causing any deleterious effects on soil Collembola.

Total metal analysis (Table 2) from the five sites showed that Ladymoor had the highest

Table 2. Total and water soluble metal concentrations ( $\mu\text{g g}^{-1}$ ), and pH (pH-H<sub>2</sub>O) of the four replicate soil cores ( $\pm$  SE) from the five sites in Wolverhampton, sampled in the Spring of 1999 (pooled sample of 0–10 cm core)

Wolverhampton Site	Cd (total)	Cu (total)	Pb (total)	Zn (total)	pH
EPG	0.9 (0.2)	279 (31)	233 (9)	702 (81)	6.0 (0.2)
EPW	2.2 (0.9)	295 (64)	220 (39)	656 (143)	6.0 (0.1)
PPW	0.7 (0.2)	128 (6)	309 (24)	437 (35)	6.3 (0.1)
BGW	0.4 (0.2)	115 (3)	233 (33)	497 (18)	7.9 (0.1)
L	14.2 (2.8)	1226 (352)	1191 (103)	7907 (1874)	7.5 (0.2)
Background levels (total)	0.01–0.5	20–30	10–40	10–300	
Wolverhampton Site	Cd (H <sub>2</sub> O soluble)	Cu (H <sub>2</sub> O soluble)	Pb (H <sub>2</sub> O soluble)	Zn (H <sub>2</sub> O soluble)	
EPG	Below detection	9.4 (5.1)	0.5 (0.1)	10.3 (2.0)	
EPW	Below detection	3.7 (0.5)	0.6 (0.2)	9.7 (1.1)	
PPW	Below detection	1.2 (0.1)	2.6 (0.3)	5.3 (0.4)	
BGW	Below detection	1.6 (0.8)	0.7 (0.1)	3.0 (0.9)	
L	Below detection	6.4 (0.7)	5.3 (0.2)	18.4 (3.4)	

The range of background levels for metals in non-polluted soils were taken from Merian (1962). See Table 1 for site abbreviations.

levels of Cd, Cu, Pb and Zn (ANOVA  $F_{4,15} = 7.29\text{--}20.42$ , Fishers pairwise comparisons  $p < 0.01$ ). Concentrations of Co, Cr, Fe and Ni were close to background levels as reported in Merian (1962) and are not shown. Metals were positively correlated across the sites (Pearsons correlation,  $r > 0.608$ ). For this reason, and because Zn is the metal most likely to be causing an effect on *F. candida* (Fountain and Hopkin, 2001; Hopkin and Spurgeon, 2001; Lock and Janssen, 2001) the results for Zn only will be displayed throughout the rest of this paper. Within each site the total Zn levels were higher for Ladymoor than the other sites at every 2 cm interval in the soil profile (ANOVA  $F_{4,15} = 8.73\text{--}16.71$ , Fishers pairwise comparisons  $p < 0.01$ , Fig. 1). The distribution of Zn throughout the soil profile was dependent upon the site (Fig. 1). At EPG, EPW and Bilston the Zn levels tended to decrease with increased depth (ANOVA  $F_{4,15} = 3.02\text{--}5.81$ , Fishers pairwise comparisons  $p < 0.05$ ). Peascroft and Ladymoor had no significant differences in Zn levels within the soil profile (ANOVA  $F_{4,15} = 1.35\text{--}2.03$ , Fishers pairwise comparisons  $p > 0.05$ ).

Unlike the total metal concentrations, no increase or decrease with soil depth was evident with the water soluble Zn levels in the soil cores, and these data are not shown. Water soluble zinc levels (0–10 cm amalgamated samples, Table 2) were less than 1.5% of the total concentrations.

Ladymoor soil had the highest water soluble Zn levels in comparison to the other sites.

The percentage OM (Fig. 2) was highest at Ladymoor followed by EPW > Bilston > Peascroft > EPG. OM is significantly higher at Ladymoor than EPG, Peascroft and Bilston. The upper levels of the soil (0–4 cm) had a higher OM than deeper levels (ANOVA  $F_{4,15} = 2.03\text{--}13.05$ , Fishers pairwise comparisons  $p < 0.01$ ), except at the Ladymoor site which had a high OM content throughout the profile. Soil pH (pH-H<sub>2</sub>O) at all sites was close to neutral (Table 2).

#### Species data

A total of 6040 individual springtails were identified to species. A list of the 38 species of Collembola found at the five sites during the Spring, Summer and Autumn of 1999 is shown in Table 3.

Abundance of Collembola at the sites varied from 1850 ( $\pm$  665) individuals  $\text{m}^{-2}$  at the Bilston site in the Spring, to 70,500 ( $\pm$  20,360) individuals  $\text{m}^{-2}$  at the Ladymoor site in the Autumn. There were only four species that were common to all five sites, namely *Paratullbergia callipygos* (PT CAL), *Isotoma notabilis* (IS NOT), *Isotomurus palustris* (IR PAL) and *Sminthurinus elegans* (SN ELE). Some species were only found at one site. At EPG, *Friesea mirabilis* (FR MIR), *Folsomia bisetosa* (FO BIS), *Proisotoma minuta* (PI MIN) and *Vertagopus arboreus* (VE ARB) were species exclusive to this

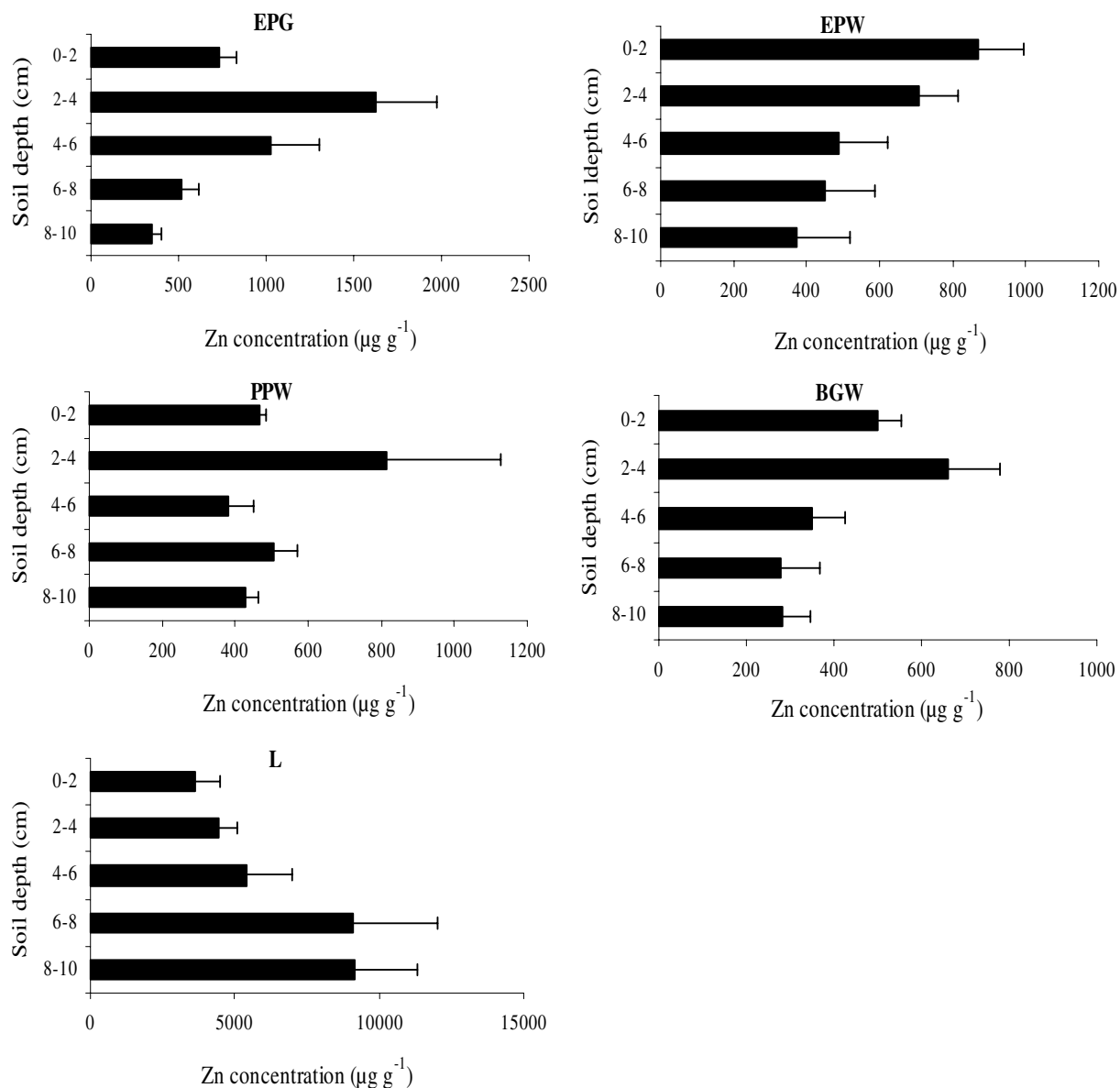


Figure 1. Histograms showing the total Zn concentrations ( $\mu\text{g g}^{-1} \pm \text{SE bars}$ ) in the soil profile, taken from four replicates at 2 cm intervals from 0–10 cm. See Table 1 for site abbreviations.

site. Only one species was exclusive to EPW namely, *Cryptopygus bipunctatus* (CR BIP). *Kalaphorura burmeisteri* (KA BUR), *Entomobrya multifasciata* (EN MUL), *Folsomia spinosa* (FO SPI), *Isotomiella minor* (IM MIN) and *Oncopodura crassicornis* (OC CRA) were the species only present at Peascroft, and *Ceratophysella denticulata* (CE DEN), *Ceratophysella gibbosa* (CE GIB) and *Dicyrtomina minuta* (DM MIN) were only

found at Ladymoor. All of the species at Bilston were found in at least one of the other sites.

The data from all five sites fitted the lognormal model ('goodness of fit' tests on species distributions  $p > 0.05$ ). For some soil cores, species distributions could not be fitted for the Spring and Summer months because counts of springtails were too low for analysis. Therefore, the following analysis was applied to the Autumn data only.

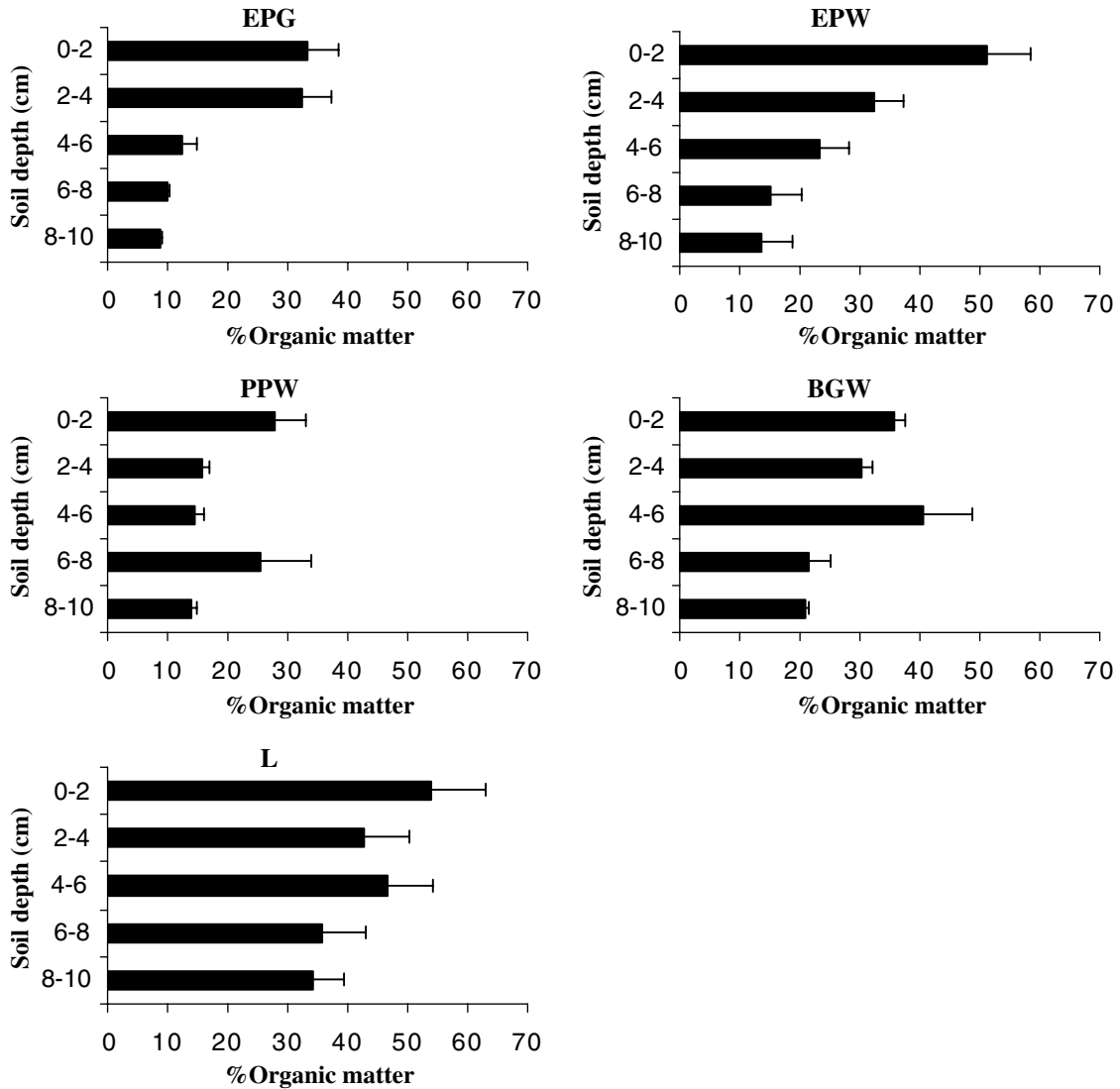


Figure 2. Histograms showing the percentage OM of total soil ( $\pm$ SE bars) in the soil profile, taken from four replicates at 2 cm intervals from 0 to 10 cm, for the five Wolverhampton sites. See Table 1 for site abbreviations.

When commonly used species indices were applied, it became clear that diversity varied for a site depending on the index used (Fig. 3). With Shannon–Weiner, Bilston had a significantly higher diversity than Ladymoor and Peascroft (ANOVA  $F_{4,14} = 3.67$ , Fishers pairwise comparisons  $p < 0.05$ ).

Bilston had a significantly lower diversity however, compared to EPW, Peascroft and Ladymoor when the Simpson index was used. Ladymoor had a significantly increased diversity

than the other four sites (ANOVA  $F_{4,15} = 9.39$ , Fishers pairwise comparisons  $p < 0.05$ ).

For the Margalef index, fewer differences were obtained between sites. EPG had a significantly higher diversity than EPW, and Ladymoor had a higher diversity than Peascroft and EPW (ANOVA  $F_{4,15} = 3.68$ , Fishers pairwise comparisons  $p < 0.05$ ).

The alpha index showed that Bilston was higher in diversity than both EPW and Ladymoor, and that EPW had a lower diversity than Peascroft

Table 3. Species list of Collembola present in the Wolverhampton soils (see Table 1 for site abbreviations), extracted from four soil cores at each site in the Spring, Summer and Autumn (pooled data)

Species name	Abbreviation	EPG	EPW	PPW	BGW	L
<i>Brachystomella parvula</i>	BR PAR	✓			✓	
<i>Ceratophysella denticulata</i>	CE DEN					✓
<i>Ceratophysella gibbosa</i>	CE GIB					✓
<i>Xenylla boernerii</i>	XL BOE	✓				✓
<i>Friesea mirabilis</i>	FR MIR	✓				
<i>Friesea truncata</i>	FR TRU				✓	✓
<i>Neanura muscorum</i>	NN MUS		✓	✓		✓
<i>Kalaphorura burmeisteri</i>	KA BUR			✓		
<i>Protaphorura armata</i>	PR ARM	✓				✓
<i>Mesaphorura krausbaueri</i>	MS KRA	✓			✓	✓
<i>Paratullbergia callipygos</i>	PT CAL	✓	✓	✓	✓	✓
<i>Paratullbergia macdougalli</i>	PT MAC				✓	✓
<i>Entomobrya multifasciata</i>	EN MUL			✓		
<i>Lepidocyrtus lanuginosus</i>	LE LAN		✓	✓		✓
<i>Pseudosinella alba</i>	PS ALB		✓			✓
<i>Heteromurus nitidus</i>	HT NIT	✓		✓		✓
<i>Orchesella villosa</i>	OR VIL	✓	✓	✓	✓	
<i>Cryptopygus bipunctatus</i>	CR BIP		✓			
<i>Cryptopygus thermophilus</i>	CR THE	✓			✓	✓
<i>Folsomia candida</i>	FO CAN				✓	✓
<i>Folsomia fimetaria</i>	FO FIM	✓				✓
<i>Folsomia quadrioculata</i>	FO QUA		✓	✓		
<i>Folsomia spinosa</i>	FO SPI			✓		
<i>Folsomia bisetosa</i>	FO BIS	✓				
<i>Isotoma anglicana</i>	IS ANG	✓	✓		✓	✓
<i>Isotoma notabilis</i>	IS NOT	✓	✓	✓	✓	✓
<i>Isotoma tigrina</i>	IS TIG		✓			✓
<i>Isotomiella minor</i>	IM MIN			✓		
<i>Isotomodes productus</i>	IT PRO	✓			✓	✓
<i>Isotomurus palustris</i>	IR PAL	✓	✓	✓	✓	✓
<i>Proisotoma minuta</i>	PI MIN	✓				
<i>Vertagopus arboreus</i>	VE ARB	✓				
<i>Oncopodura crassicornis</i>	OC CRA			✓		
<i>Megalothorax minimus</i>	MG MIN		✓	✓	✓	
<i>Deuterostminthurus pallipes</i>	DE PAL		✓	✓	✓	✓
<i>Dicyrtomina minuta</i>	DM MIN					✓
<i>Sminthurinus elegans</i>	SN ELE	✓	✓	✓	✓	✓
<i>Sphaeridia pumilis</i>	SP PUM	✓	✓		✓	✓

(ANOVA  $F_{4,15} = 3.66$ , Fishers pairwise comparisons  $p < 0.05$ ).

A simple count of the number of species at each site (species richness) revealed that Ladymoor had a higher number of species than both EPW and Peascroft. Peascroft also had a lower number of species than EPG (ANOVA  $F_{4,15} = 5.10$ , Fishers pairwise comparisons  $p < 0.01$ ).

Two indices that measure evenness/dominance are the Shaneven and the Berger–Parker dominance index, respectively, and these show inverse results. Hence, Ladymoor had a significantly lower

evenness than the other four sites (ANOVA  $F_{4,15} = 9.66$ , Fishers pairwise comparisons  $p < 0.05$ ), and a significantly higher dominance than EPG and Bilston (ANOVA  $F_{4,15} = 3.74$ , Fishers pairwise comparisons  $p < 0.05$ ).

The mean abundance of dominant species varied considerably between sites. At Ladymoor, the dominant species, *I. palustris*, was present at 38,250 m<sup>-2</sup> ( $\pm 10,678$ ). This figure contrasts with soil from Peascroft where the dominant species *Folsomia quadrioculata* was present at only 2000 individuals m<sup>-2</sup> ( $\pm 1243$ ).

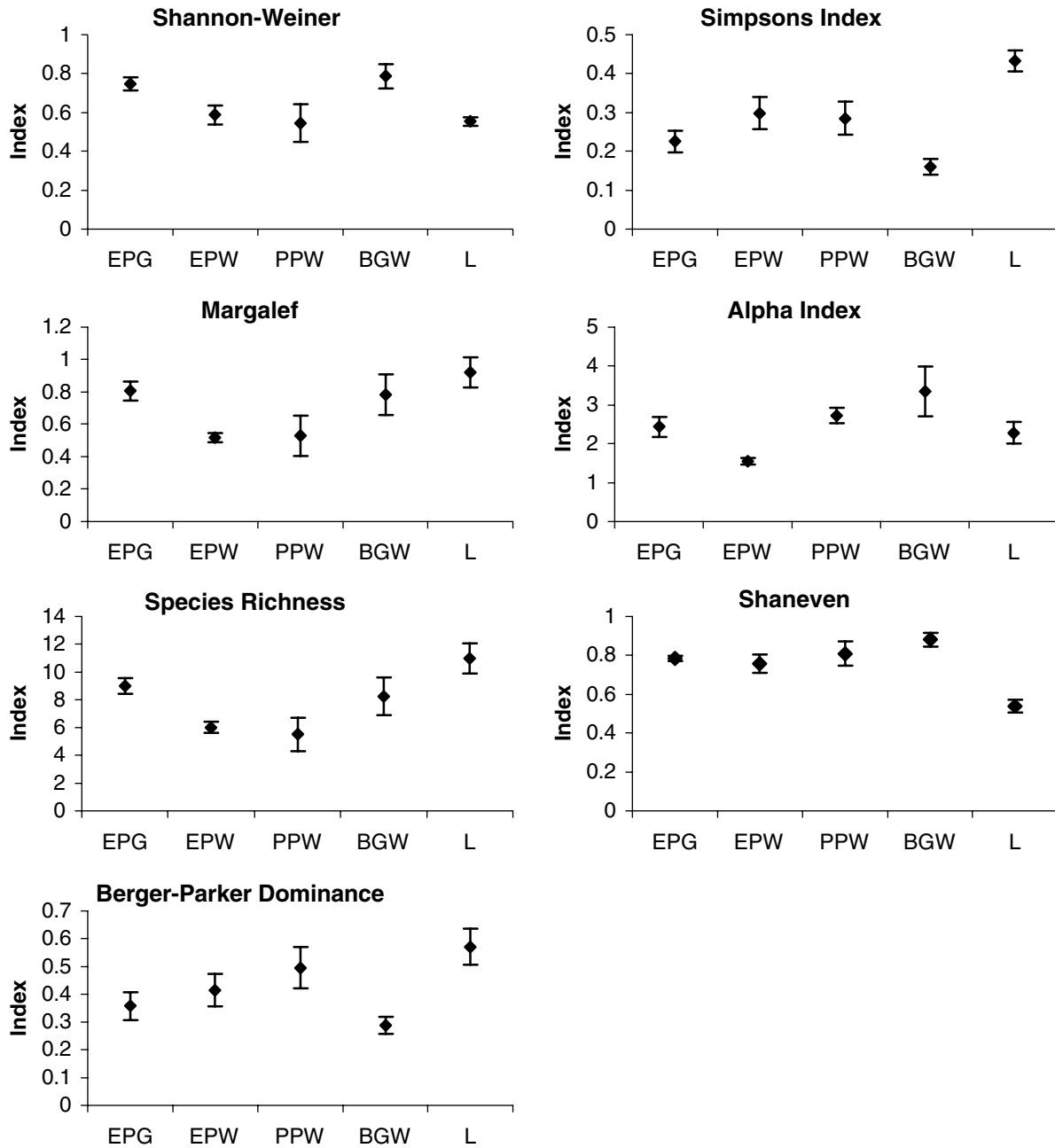


Figure 3. Species indices (Shannon–Weiner, Simpson Index, Margalef Index, Alpha Index, Species Richness, Shaneven and Berger-Parker Dominance) for the five Wolverhampton sites (mean of four soils cores  $\pm$ SE bars, see Table 1 for site abbreviations).

The dominant species (species represented by greater than 10% of the total number) differed depending on the site. At EPG, they may be considered as: *Isotomodes productus* (IT PRO, 37%),

*Cryptopygus thermophilus* (CR THE, 24%), *Iso-toma anglicana* (IS ANG, 17%) and *S. elegans* (SN ELE, 11%); at EPW, *I. notabilis* (IS NOT, 37%), *S. elegans* (SN ELE, 16%), *I. anglicana* (IS ANG,

15%), *Lepidocyrtus lanuginosus* (LE LAN, 12%) and *Isotoma tigrina* (IS TIG, 10%); at Peascroft, *F. quadrioculata* (FO QUA, 40%), *I. notabilis* (IS NOT, 17%) and *L. lanuginosus* (LE LAN, 14%); at Bilston, *I. anglicana* (IS ANG, 22%), *Brachystomella parvula* (BR PAR, 15%), *I. notabilis* (IS NOT, 14%) and *S. elegans* (SN ELE, 13%) and at Ladymoor, *I. palustris* (IR PAL, 54%) and *I. notabilis* (IS NOT, 24%), only. Ladymoor had the most species (24), followed by EPG (19), Bilston and Peascroft (16) and EPW (15) for Spring, Summer and Autumn samples combined (Table 3). Rank abundance plots (Fig. 4), plotted on a log scale, provide a clearer picture of this with Ladymoor showing a higher dominance and more species when compared to the other sites.

Frequency distribution plots showed a skewed distribution (data not shown). The most abundant class varied from site to site, but was never the class represented by just one individual per species for any of the sites, giving more support to the lognormal distribution for these communities. The lognormal data were plotted onto a geometric scale ( $2\times$  scale) against the cumulative number of species percentage (Fig. 5). Ladymoor had a higher number of geometric classes and a shallower line than the other sites.

#### *Folsomia candida* exposure test

The results of the soil test, where *F. candida* was exposed to Wolverhampton soil for 4 weeks are shown in Fig. 6. The pH (pH-H<sub>2</sub>O) of the soil changed by less than 0.9 from the beginning to end of the experiment and the water content decreased by less than 2.7% for all of the containers. The number of adults that survived at the end of the exposure period was significantly lower in Ladymoor (45%), Peascroft (56%) and Bilston (57%) than in the EPG (72%) and EPW (75%) soil (ANOVA  $F_{4,70} = 6.18$ , Fishers pairwise comparisons  $p < 0.01$ , Fig. 6). Juveniles were also less abundant in Ladymoor (456), Bilston (727) and Peascroft (927) soil. Additionally, Ladymoor and Bilston had significantly fewer juveniles than Peascroft (ANOVA  $F_{4,70} = 17.77$ , Fishers pairwise comparisons  $p < 0.01$ ). The mean number of juveniles per test container in EPG and EPW was 1687 and 1450, respectively.

## Discussion

### *Species data*

The number of species of Collembola found at each site in Wolverhampton was 19 (EPG), 15 (EPW), 16 (PPW), 16 (BGW) and 24 (Ladymoor).

As in other studies (e.g. Chagnon et al., 2000), the soils in Wolverhampton displayed a high number of species with a low dominance and a low number of species with a high dominance. Haimi and Siirapietikainen (1996) found 22, 18 and 17 species of Collembola 8, 2 and 0.5 km from a smelting works, respectively. The number of Collembola was also significantly lower near to the smelter ( $<2000 \text{ m}^{-2}$  compared to  $>6000 \text{ m}^{-2}$  at 8 km from the smelter). Hågvar and Abrahamsen (1990) also found that species number decreased with increasing Pb concentration along a gradient. It is difficult to determine why there are differences in species numbers in the Wolverhampton soils, as they are very different sites, both in anthropogenic use and vegetation composition e.g. EPG is regularly disturbed by mowing and trampling, as it is a recreational field. In comparison, Ladymoor is a nature reserve (in spite of its polluted soil) and is relatively undisturbed by recent anthropogenic activity. The older a site becomes, the higher the species richness and community diversity may become. This effect cannot be ruled out as being the causes of differences in species number or diversity. Industrial use of the Ladymoor site ceased in 1920 and so it has been left relatively unmanaged for over 80 years. This may explain its high species richness compared to the other sites.

Changes in vegetation have been shown to change the composition of Collembola (Fox, 1967; Moore et al., 1984; Fratello et al., 1985; Wardle et al., 1993) and this factor needs to be considered in field studies using different sites. A decreased vegetation canopy can change the microclimate by affecting the temperature and moisture of soil (House et al., 1987). The addition of organic fertilizer to industrial wasteland increases vegetation cover/plant complexity, which can increase species richness and abundance of soil animals (Kampichler et al., 1999).

The abundance of Collembola in studies by other workers is variable. Ranges from  $10^3$  to over

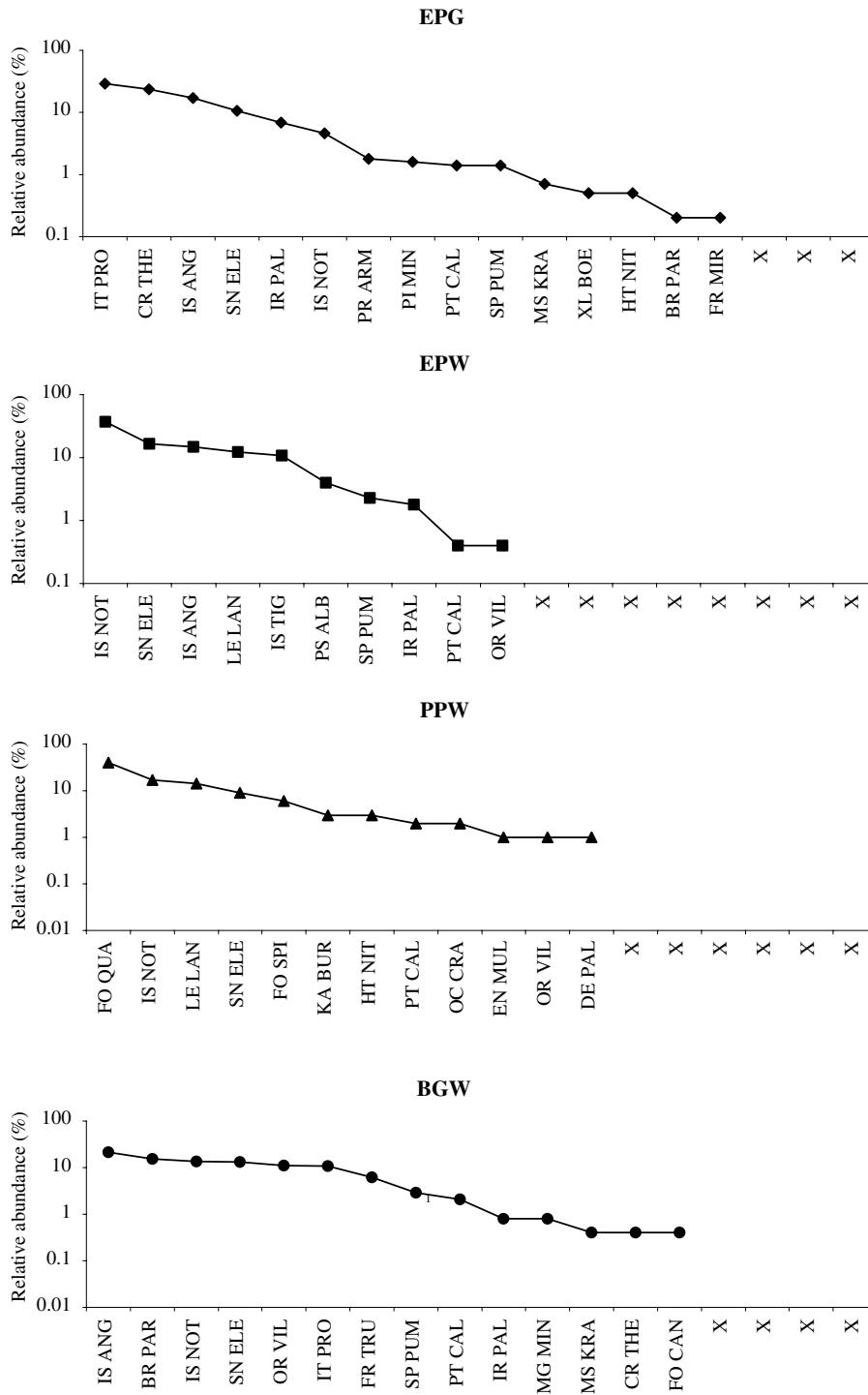


Figure 4. Rank abundance plots of Collembola from the five Wolverhampton sites plotted against the percentage relative abundance of species ( $n = 4$ ). For the site and species abbreviations see Tables 1 and 3, respectively.

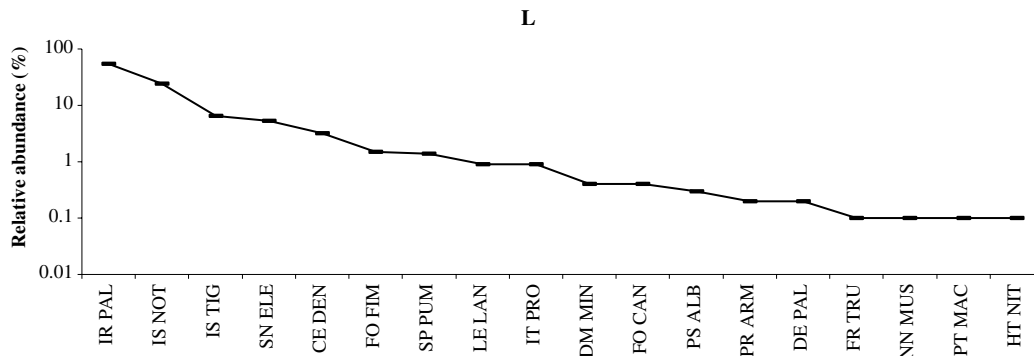


Figure 4. Continued.

$10^5$  individuals  $m^{-2}$  have been reported (Davis, 1965; Burnett, 1968; Curry, 1969; Kelly and Curry, 1985; Chakravorty and Joy, 1990; Kuznetsova and Potapov, 1997; Rodgers, 1997; Bardgett and Cook, 1998; Mebes and Filser, 1998; Russell and Alberti, 1998; Axelsen and Kristensen, 2000).

Total abundances (individuals  $m^{-2}$ ) in this study for the five sites in the Autumn were (mean ( $\pm$  SE)) 21,650 (4496) for EPG, 13,900 (4336) EPW, 4950 (1956) Peascroft, 12,050 (3742) Bilston and 70,500 (20,360) Ladymoor, and are within the ranges found by other workers.

Densities of Collembola near to a smelting works were 6117, 14,018 and 15,398  $m^{-2}$  at 1, 6 and 40 km from the smelter, respectively (Strojan, 1978). However, in field studies by Bengtsson and Rungren (1988), species richness, density and diversity were not related to an increased Cu level encountered near to a brass mill. Bruce et al. (1999) also found that abundance was not changed

by metal contaminated soils. Thus, there is not necessarily an obvious relationship between total Collembola abundance and soil contamination.

Bruus Pedersen et al. (1999) reported a decrease in the Shannon–Weiner index for Collembola with increasing Cu concentrations. However, comparisons for polluted sites using species indices are unreliable. If we compare indices from data in this study (Fig. 3), Ladymoor has the highest diversity using the Simpson and the Margalef indices, but Bilston has the highest diversity using the Shannon–Weiner and alpha index. This is because the former indices are more weighted toward the number of species in each sample, whereas the latter are weighted towards the number of individuals. Shannon–Weiner also puts an emphasis on rare species, whereas the Simpson index emphasises the common species (Krebs, 1999). In contaminated sites, pollution sensitive species may decrease in number without disappearing and

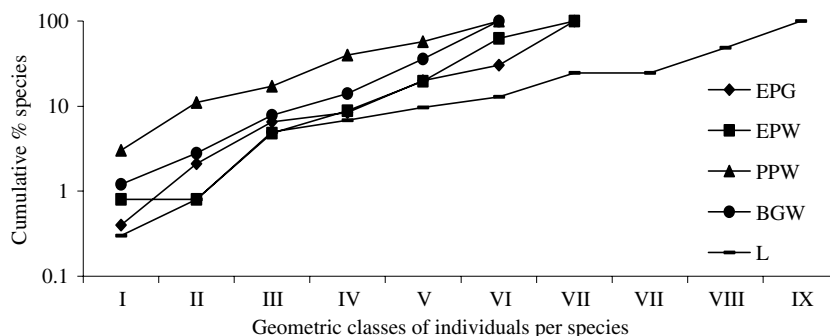


Figure 5. Lognormal plots ( $n = 4$ ) of Collembola from five Wolverhampton sites. Cumulative species plotted against a geometric scale ( $\times 2$  scale) of individuals per species. For the site abbreviations see Table 1.

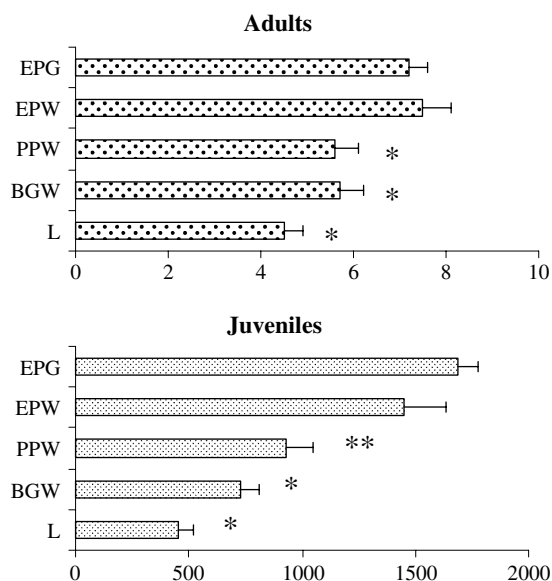


Figure 6. Number (mean of 16,  $\pm$ SE bars) of adults surviving and juveniles produced at the end of a 4 week exposure experiment in the soil from five Wolverhampton sites (ANOVA  $F_{4,70} = 6.18-17.77$ , Fishers pairwise comparisons  $p < 0.01$ ). \* and \*\* denote significant differences from EPG and EPW, \*\* also shows significant differences from PPW.

increase the species index (Cortet et al., 1999). Ladymoor has a low evenness ( $0.54 \pm 0.034$ ) and hence a high dominance compared to the other sites. Low evenness values have been found to be characteristic of disturbed sites (Lauga-Reyrel and Deconchat, 1999) and such sites, e.g. agricultural fields, have lower diversity values than wasteland (undisturbed) sites (Edwards and Thompson, 1973; Cancela Da Fonseca and Sarkar, 1996). However, a contaminated site with a high diversity may not be as functionally competent as a "clean" site, because the metal tolerant species that have replaced the metal sensitive species may not fulfil the same roles in the ecosystem. Also, species indices are not species sensitive and take no account of indicator species (Van Straalen, 1997).

Ladymoor is dominated by just two species (a characteristic of polluted sites, see Table 4). A few tolerant species increase in abundance possibly due to reduced competition and reduced predation from sensitive species of soil fauna, whilst common species manage to survive at a low abundance. *I. notabilis* has been shown to be dominant within the zone of emission of a Fe and steel works (3.5 km),

whilst samples taken 9 km from the smelter had a higher diversity and were described as the transitional stage between the clean and polluted site (Kuznetsova and Potapov, 1997). At the Gusum metal polluted site in Sweden, *Folsomia fimetarioides* is one of the few dominant species close to the plant and as the metal concentration further from the site decreases, *F. fimetarioides* abundance also decreases (Sjögren, 1986). The advantage of *F. fimetarioides* over other species is its preference for metal tolerant fungi (Tranvik and Eijsackers, 1989). This species has been suggested as an indicator for metal pollution, but was not present at the Wolverhampton sites in this study (Table 4).

Species assemblages in polluted soils may change due to quantitative and qualitative changes in food, increased bioavailability of metals, avoidance of contamination by migration, and species-specific detoxification abilities. Some Collembola are specialist feeders and have preferences over which species of fungi they consume (Ponge, 1991). The metal tolerant fungus, *Paecilomyces farinosus*, is protein rich. Therefore there may be a trade-off between high quality food and metal toxicity to Collembola (Bengtsson et al., 1985; Bengtsson and Rungren, 1988; Bengtsson et al., 1994; Hopkin, 1994; Martikainen et al., 1998). Filser et al. (1995, 2000) suggested that Cu decreased or changed the microbial flora, which decreased the species number and abundance of Collembola when Cu was added to the soil.

The four dominant species at EPG have not been documented (Table 4) as metal tolerant species. At EPW, *I. notabilis* is dominant with 39% of the total number of individuals, but the views on this species as an indicator of metal pollution are conflicting (Lübber, 1989; Nüss, 1994; Haimi and Siirapietikainen, 1996; Kuznetsova and Potapov, 1997, see Table 4) as are references for *F. quadriculata* (Bengtsson and Rungren, 1988; Hågvar and Abrahamson, 1990; Filser et al., 1995; Bruus Pedersen et al., 1999), the dominant species at Peascroft. Ladymoor is dominated by *I. palustris* (54%), which has been shown to be predominant in metal contaminated soils (Cole et al., 2001). Bilston is dominated by *I. anglicana*; again conflicting reports exist as to whether this species is unaffected or predominant in metal contaminated sites (Bruce et al., 1999; Cole et al., 2001). *C. denticulata* was only found at Ladymoor and is also considered to

Table 4. Status of Collembola species in the most metal contaminated field sites reported in the literature

Increased or predominant species	Unaffected species	Decreased species	Absent species	Reference
<u>Ceratophysella denticulata</u>		<u>Cryptopygus thermophilus</u>		Cole et al. (2001) Lübben (1989)
<i>Folsomia dovrensis</i> <i>Folsomia fimetarioides</i>				Haimi and Siirapietitainen (1996) Sjögren (1986), Bengtsson and Rungren (1988), Tranvik and Eijsackers (1989), Haimi and Siirapietitainen (1996)
<u>Folsomia quadrioculata</u>		<u>Folsomia quadrioculata</u>	<u>Folsomia quadrioculata</u>	Bengtsson and Rungren (1988), Hågvar and Abrahamsen (1990), Filser et al. (1995), Bruus Pedersen et al. (1999) Filser and Hölscher (1997)
<i>Folsomia manolachei</i>			<u>Friesea mirabilis</u>	Bengtsson and Rungren (1988) Bruce et al. (1999), Cole et al. (2001)
<u>Isotoma anglicana</u>	<u>Isotoma anglicana</u>			
<i>Isotoma notabilis</i>		<u>Isotoma notabilis</u>		Lübben (1989), Nüss (1994), Haimi and Siirapietitainen (1996), Kuznetsova and Potapov (1997)
<i>Isotoma olivacea</i>		<i>Isotoma viridis</i> <i>Isotomiella minor</i>		Hågvar and Abrahamsen (1990) Bruce et al. (1997), Cole et al. (2001)
<u>Isotomurus palustris</u>		<i>Lepidocyrtus cyaneus</i> <i>Mesophorura sp.</i>		Bengtsson and Rungren (1988), Filser et al. (1995) Cole et al. (2001) Bruce et al. (1997), Cole et al. (2001)
<i>Mesophorura sp.</i> <u>Mesophorura krausbauri</u> <i>Neelus minimus</i>		<i>Neelus minimus</i>		Lübben (1989), Bruce et al. (1999) Filser et al. (1995, 2000) Bruce et al. (1999)
<i>Onychiurus armatus</i>	<u>Orchesella villosa</u>			Bengtsson and Rungren (1988) Hunter et al. (1987)
		<u>Pseudosinella alba</u>		Filser et al. (2000)
<i>Sminthurinus aureus</i>	<i>Sminthurinus aureus</i>	<i>Sminthurinus aureus</i>		Lübben (1989), Kuznetsova and Potapov (1997), Bruus Pedersen et al. (1999)
<i>Tullbergia sp.</i>				Bengtsson and Rungren (1988) Filser and Hölscher (1997)
<i>Willemia anophthalma</i>		<i>Willemia intermedia</i>		Lübben (1989)

Species underlined were found in this study

be predominant in metal contaminated soils (Cole et al., 2001).

Lognormal distributions occur where the most abundant class is not represented by one species. This type of distribution will produce a straight line if plotted on a geometric scale of individuals per species (Fig. 5). Grey (1981) describes how an unpolluted benthic marine ecosystem has 5–6 geometric classes, with the polluted phase having

14–15 classes. Grey (1981) also hypothesised how both of these communities are at equilibrium and in the polluted site most species have become rare and a few have become dominant. Similar results have been observed for Collembola and Oribatei (Hågvar, 1994). Communities under stress show an increase in a few dominant species and sensitive species have a low abundance. These effects have been observed with stresses such as acid,

metals and ploughing (Hågvar, 1994). Haimi and Siirapietikanen (1996) have also shown that soil animals can maintain their populations at low levels in highly contaminated soils. Ladymoor has 10 geometric classes compared to the 6 or 7 classes of the other four sites and the line relating these to cumulative number of species is shallower (Fig. 5).

#### *Folsomia candida* exposure test

Field contaminated soil has been shown to have a significantly lower effect on Collembola than laboratory contaminated (spiked) soil with the same metal concentrations. In experiments using “spiked” and field soil contaminated with Zn, the “spiked” soil had a strong effect on *F. candida* reproduction whereas the field soil showed no effect at the same concentration (Smit and Van Gestel, 1996, 1998). As with this study, it was also shown that differences in water soluble metals did not fully explain the differences between the soils (Scott-Fordsmand et al., 1998). Aged soils are leached of available metals by rainwater, while an increase in sorption of remaining residues results in a reduced bioavailability (Smit et al., 1997). In this study all of the sites had fewer parent *F. candida* surviving than is recommended for the control of the standard test (80%) using OECD soil (Wiles and Krogh, 1998). The highest reproduction (juveniles per test container) in this experiment was seen for soils from EPG (1687) and EPW (1450) and is higher than reproduction typically seen in the controls of experiments using the standard OECD soil ( $n = 500$ – $1000$ ) Krogh and Bruus Pedersen, 1995; ( $n = 797$ ) Sandifer and Hopkin, 1996, 1997). Once again Ladymoor appears to have the most detrimental soil for *F. candida*, with lower numbers of adults and juveniles than the other sites (Fig. 6). The low reproductive output in the Bilston and Peascroft soils must be due to another factor as metal levels are not high enough to cause a significant effect. For example Crouau et al. (1999) found that the peak reproduction of *F. candida* was at pH 5.2, with significantly fewer juveniles in more alkaline soils (pH 6.9). Therefore, in the contaminated Wolverhampton soils it is likely that there are other contributing factors to the survival and reproduction of the test collembolan, *F. candida*.

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