

# Colonization of heavy metal-polluted soils by Collembola: preliminary experiments in compartmented boxes

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1	Colonization of heavy metal polluted soils by Collembola: preliminary
2	experiments in compartmented boxes
3	
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5	
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8	
9	Abstract
10	
11	Two-week laboratory experiments were carried out in plastic boxes separated in two connected
12	compartments filled with a neutral soil (mull humus) at pH 7.7 and an acid soil (moder humus) at pH
13	4.3, containing their original faunas. Migration of Collembola from one compartment to the other was
14	allowed through a perforated wall. The mull was contaminated with three concentrations of lead (as
15	lead acetate) at 50, 6,000 and 60,000 ppm. When combined with moder in the adjacent compartment,
16	6 times more individuals and 5 times more species were observed in the mull at the highest
17	concentration applied, compared to mull combined with itself. Parisotoma notabilis proved to be highly
18	sensitive to lead, and shifted to the moder compartment even at the lowest concentration. Densities of
19	other mull species such as Pseudosinella alba were affected by medium to high concentrations of lead
20	but these species did not move to the moder soil despite their high motility. Acidophilic species living
21	in moder only, such as Willemia anophthalma, Xenylla tullbergi, Proisotoma minima and Xenylla
22	tullbergi, colonized contaminated mull treatments with densities increasing with lead concentrations
23	but the result of this process was erratic. Folsomia manolachei was present in both humus forms but
24	was much more abundant in the moder. This species colonized mull at medium to high lead
25	concentrations, where it restored totally or partly its original abundance in the uncontaminated mull.
26	These results suggested differences between mull and moder populations of Folsomia manolachei.
27	

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1 *Keywords:* Collembola, Acidophily, Heavy metals, Migration, Sensitivity.

2

#### 3 **1. Introduction**

4

5 The sensitivity of Collembola (Hexapoda) to heavy metals has been the subject of recent 6 investigations, pointing on the heritability of resistance in populations submitted to long-term 7 contamination of soils (Posthuma, 1990; Posthuma et al., 1993; Tranvik et al., 1994). Several studies 8 have been carried out on collembolan communities, showing that shifts in species composition 9 occurred under the influence of heavy metal contamination, together with a decrease in biodiversity 10 (Hågvar and Abrahamsen, 1990; Filser and Hölscher, 1997; Bruus Pedersen et al., 1999).

11

The sensitivity of different species of Collembola to soil acidity has been recognized for a long time (Gisin, 1943; Mertens, 1975; Hågvar and Abrahamsen, 1984; Loranger et al., 2001). Experiments with artificial acid rain have shown that species living only in acid soils such as *Willemia anophthalma*, *Micranurida pygmaea* and *Mesaphorura yosii* were favoured by the application of sulphuric acid. Other species, present in acid soils but more abundant in neutral soils, such as *Parisotoma notabilis*, were eliminated by the same experimental conditions (Bååth et al., 1980; Hågvar and Kjøndal, 1981; Hågvar, 1984).

19

20 The presence of high concentrations of aluminum, iron and other metals in the soil solution 21 when soils are very acid (Bergkvist, 1987; Lundström et al., 2000), and the increased ecological 22 effects of heavy metals at low pH (Crommentuijn et al., 1997), led us to postulate that tolerance to heavy metals and tolerance to acidity could be ecologically related. Specifically, animals living in acid 23 24 soils, either at the species or the sub-species level, could be more tolerant to heavy metals than 25 animals commonly living in neutral or weakly acidic soils. The threshold of pH 5, below which changes in collembolan species composition have been recorded (Ponge, 1983; Ponge, 1993; Ponge, 2000a), 26 27 is also a threshold under which aluminum passes into the soil solution as a metal cation and may exert severe toxicity, mostly by negatively interacting with phosphorus metabolism (Clarkson, 1969; James 28 and Riha, 1984; Berggren et al., 1990). Given a common detoxication strategy in Collembola and 29 30 other Hexapoda, i.e. by periodically renewing the midgut epithelium (Joosse and Buker, 1979; Hopkin,

1995), it can be hypothesized that species living in naturally acidic soils (and thus thought tolerant or
 resistant to free metallic forms) are able to colonize neutral or weakly acidic soils contaminated with
 heavy metals as a consequence of human activities such as mining, agriculture and industry.

4

It is intended to test this hypothesis in the long-term by inoculating acid humus profiles, with their complete acido-tolerant collembolan communities, into metal-contaminated sites. Prior to undertaking field experiments, a preliminary study was conducted in order to verify whether Collembola living in acid soils are able to leave their original habitat to colonize a neutral soil polluted with heavy metals.

10

#### 11 2. Materials and Methods

12

13 Short-term experiments were conducted in experimental boxes where animals were free to 14 move between compartments, one filled with an acid soil (moder humus), and another filled with a neutral soil (mull humus) treated with lead acetate at different concentrations. Two soils were chosen, 15 16 a Dysmoder, characterized by the accumulation of organic matter in the form of small enchytraeid 17 faeces, and an Eumull, characterized by a rapid mixing of organic matter to mineral matter through 18 earthworm activity (Brêthes et al., 1995; Ponge, 1999). These soils contain very different collembolan 19 communities (Ponge, 1993; Chagnon et al., 2000). Lead acetate was chosen as the metal salt in order 20 to avoid possible toxic effects of the anion. Given the rapid disappearance of acetate in a biologically 21 active soil (Verschueren, 2001), most lead becomes rapidly complexed by soil organic matter (Sarret 22 et al., 1997; Balabane et al., 1999).

23

Topsoil horizons, with their complete original fauna, were collected in May 2000. The Eumull material (pH 7.7) was collected in a rendzina soil in the park adjacent to the laboratory (Brunoy, 20km south of Paris) under oak (*Quercus robur* L.) and hornbeam (*Carpinus betulus* L.), with a ground flora of ivy (*Hedera helix* L.) and dog's mercury (*Mercurialis perennis* L.). The sparse litter horizon was discarded, together with aerial parts of ground vegetation, and the top 10cm of the A horizon were collected over a square meter area. The Dysmoder material (pH 4.3) was collected over the same area in a pine stand (*Pinus sylvestris* L.) located in the Senart forest near the laboratory. The ground cover was mainly bramble (*Rubus fruticosus* L.) and bracken [*Pteridium aquilinum* (L.) Kuhn]. The
 superficial litter (OL horizon) was discarded, and only the OF horizon (fragmented litter) and the thick
 OH horizon (humified litter, 10cm thick) were collected. In both cases the soil was thoroughly crumbled
 by hand, most woody subterranean parts of vegetation, earthworms and stones being discarded.

5

6 Experiments were carried out in polystyrene boxes (175x115mm, 65mm deep) which were 7 divided in two compartments by a 2mm thick millboard (Isorel®) division, pierced with 4mm diameter 8 holes at a rate of 30 holes per wall. The division allowed passage by fauna but prevented physical 9 contact between the two soils. Eumull was treated with lead acetate/deionized water solutions at three 10 dilution rates, giving rise to three concentrations in the soil at 50, 6,000 and 60,000 ppm, referred to as 11 low, medium and high concentration, respectively. These concentrations were chosen on the basis of 12 preliminary assays. Control boxes (Eumull or Dysmoder) received deionized water only. Different combinations were established with five replicates each: 13

14

15	E0/E0	Eumull versus Eumull, without any addition of lead in the two compartments
16	E1/E1	Eumull versus Eumull, with lead at 50 ppm in the two compartments
17	E2/E2	Eumull versus Eumull, with lead at 6,000 ppm in the two compartments
18	E3/E3	Eumull versus Eumull, with lead at 60,000 ppm in the two compartments
19	E0/E1	Eumull versus Eumull, with lead at 50 ppm in only one compartment (E0/E1 =
20		unpolluted Eumull compartment, E1/E0 = polluted Eumull compartment)
21	E0/E2	Eumull versus Eumull, with lead at 6,000 ppm in only one compartment (E0/E2 =
22		unpolluted Eumull compartment, E2/E0 = polluted Eumull compartment)
23	E0/E3	Eumull versus Eumull, with lead at 60,000 ppm in only one compartment (E0/E3 =
24		unpolluted Eumull compartment, E3/E0 = polluted Eumull compartment)
25	E0/D	Eumull versus Dysmoder, without any addition of lead acetate in the Eumull (E0/D =
26		Eumull compartment, D/E0 = Dysmoder compartment)
27	E1/D	Eumull versus Dysmoder, with lead at 50 ppm in the Eumull (E1/D = Eumull
28		compartment, D/E1 = Dysmoder compartment)
29	E2/D	Eumull versus Dysmoder, with lead at 6,000 ppm in the Eumull (E2/D = Eumull
30		compartment, D/E2 = Dysmoder compartment)

1	E3/D	Eumull versus Dysmoder, with lead at 60,000 ppm in the Eumull (E3/D = Eumull
2		compartment, D/E3 = Dysmoder compartment)
3	ח/ח	Dysmoder versus Dysmoder
4	0,0	
4		
5	The ex	perimental boxes were incubated in the laboratory at 15°C in darkness during two
6	weeks. At the	end of the incubation period arthropods were extracted by the dry-funnel method. The
7	identification of	of Collembola was done to the species level under a light microscope at 400x
8	magnification.	After extraction of the microarthropods, the water pH of the dried soil was measured
9	electrometrical	ly in a 1:3 (w:w) soil:water slurry.
10		
11	Data (	pH values, abundance of species or groups of species, number of species) were
12	analysed by o	ne-way ANOVA, using boxes as replicates. For treatments where both compartments
13	were filled with	n the same soil (E0/E0, E1/E1, E2/E2, E3/E3, D/D), average values between paired
14	compartments	were used for comparisons with other treatments. When necessary data were log-
15	transformed in	order to ensure additivity of variances. Comparisons among means following ANOVA
16	were achieved	a posteriori by the SNK procedure (Glantz, 1997). When homogeneity of variances
17	could not be a	achieved through log-transformation of the data, non-parametric Mann-Whitney rank
18	tests were perf	ormed in place of ANOVA (Glantz, 1997).
19		
20	3. Results	
21		
22	3.1. Compariso	on between Eumull and Dysmoder populations

A total of 37 collembolan species were found over the whole set of samples (Table 1). Among these, 17 most frequent species were selected for further analyses (Table 2).

26

Total abundance of Collembola and species composition of Eumull and Dysmoder soils used for the experiment differed to a great extent, as well as pH values (Table 2). At the end of the experiment Collembola were twice more abundant in Dysmoder compared to Eumull (Table 2, Fig. 1, compare E0/E0 to D/D), and the number of species was significantly higher (Fig. 2). Some species

1 were common to both soils, viz. Dicyrtoma fusca, Folsomia manolachei, Friesea truncata, Isotomiella 2 minor, Lepidocyrtus lanuginosus, Megalothorax minimus, Paratullbergia callipygos and Sphaeridia 3 *pumilis*. These species were called ubiquitous species. Nevertheless, among this group, some species 4 were much more abundant in one or other of the soils. For instance Folsomia manolachei was 6 times 5 more abundant in Dysmoder than in Eumull (Fig. 3), while Isotomiella minor was twice more abundant in Eumull than in Dysmoder (Table 1). Among species living only in Eumull, called Eumull species, the 6 7 most frequent ones were Heteromurus nitidus, Parisotoma notabilis and Pseudosinella alba. The most 8 frequent species living only in Dysmoder, called Dysmoder species, were Micranurida pygmaea, 9 Proisotoma minima, Pseudosinella mauli, Sminthurinus signatus, Willemia anophthalma and Xenylla 10 tullbergi. It must be noticed that these features describe the two soil samples at the end of the 11 experimental period. They refer only to one square meter (the area over which the soil was sampled) 12 in each site and they include possible changes due to experimental conditions, thus they do not 13 picture the original population.

14

The efficiency of the perforated wall for allowing animals to pass from a compartment to another can be assessed by observing that *Parisotoma notabilis*, absent from the Dysmoder soil used for the experiment, was retrieved in this soil when paired to Eumull at densities equal or even higher than those of Eumull (Fig. 4). Conversely, all Dysmoder species were retrieved in the Eumull compartment when these soils were paired (Fig. 2).

20

#### 21 3.2. Experiments on Eumull alone

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23 Eumull treatments where both compartments were given the same concentration of lead 24 (E0/E0, E1/E1, E2/E2 and E3/E3) allowed us to quantify, in our experimental conditions, the short-25 term impact of lead contamination on collembolan communities. Although soil pH increased 26 significantly at the highest treatment, the increase was small (from 7.7 to 7.8), thus no departure from 27 neutrality was observed in polluted Eumull (Table 3). The total abundance of Collembola, as well as the number of species, decreased only at high concentration (Table 3, Figs. 1 and 2). The general 28 trend of decreased abundance at high concentration only was apparent for the most abundant 29 30 species, Isotomiella minor, which comprised 57% of the collembolan population in unpolluted Eumull. 1 Lepidocyrtus lanuginosus and Heteromurus nitidus exhibited also a decrease in abundance at high 2 concentration only. Nevertheless some species did not follow this general trend. Pseudosinella alba and Sphaeridia pumilis decreased significantly in abundance at medium concentration and totally 3 4 disappeared at high concentration. Folsomia manolachei (Fig. 3) decreased progressively and 5 significantly in abundance at all three concentrations but never disappeared totally. It was the most 6 abundant species at the end of the experiment at high concentration. Parisotoma notabilis (Fig. 4) 7 decreased only at medium then nearly disappeared at high concentration. Paratullbergia callipygos 8 and Friesea truncata displayed an increase from nil to medium concentration (significant in P. 9 callipygos, not significant in *F. truncata*), then disappeared totally at high concentration.

10

11 Treatments with Eumull in both compartments but with only one of them polluted with lead 12 allowed to test the hypothesis of a possible refuge effect of unpolluted zones within a polluted site. 13 Increasing the lead concentration in the compartment adjacent to unpolluted Eumull did not affect 14 abundance and species richness of collembolan communities in the unpolluted compartment (Table 4, Figs. 1 and 2). Effects observed in the polluted compartment were similar to those observed when 15 16 both compartments were equally polluted, i.e. a decrease in abundance of species richness at high 17 concentration only (Table 5, Figs. 1 and 2). At the species level, Folsomia manolachei exhibited a 18 significant decrease in unpolluted Eumull at medium and high concentrations (Table 4, Fig. 3). In the 19 adjacent (polluted) compartment, this species exhibited a strong significant decrease at high 20 concentration only, contrary to the experiment with both compartments equally polluted, where it 21 exhibited a significant decrease as soon as low concentration was applied (Table 3, Fig. 3). No 22 change in pH was observed in the different treatments (Tables 4 and 5).

23

#### 3.3. Experiments on Eumull paired with Dysmoder

25

Treatments with Eumull combined with Dysmoder allowed us to test the hypothesis that collembolan species living in acid soils were able to colonize neutral soils polluted with heavy metals. The collembolan community of Eumull exhibited some significant changes when freely communicating with Dysmoder (Table 6, Figs. 1 and 2). In the absence of lead, a significant decrease was observed in the abundance of ubiquitous species, although no significant change occurred in the total abundance nor in the total number of species (compare E0/E0 with E0/D). At the species level, only
 *Pseudosinella alba* was affected by the presence of Dysmoder, its abundance decreasing by a factor
 of ten (Table 1). Other species did not react significantly.

4

5 After lead application, the total population of Eumull increased significantly at low and medium concentrations (compare E1/D and E2/D to E0/D). This increase, not observed when Eumull was 6 7 alone (Table 3, Fig. 1), was due to a strong increase in the abundance of ubiquitous species. At high 8 concentration, the total abundance of Collembola decreased, but was six times higher than when 9 Eumull was alone (Tables 3 and 6, Fig. 1). The improvement due to the presence of Dysmoder was 10 even more pronounced if we take into account the number of species. In the presence of Dysmoder, 11 no decrease in the number of species occurred at the high concentration of lead (Table 6, Fig. 2), 12 although this number was divided by six when Eumull was used alone. The decrease in the 13 abundance of Parisotoma notabilis observed at medium and high concentration when Eumull was 14 used alone (Table 3) was smoothed, although still significant, in the presence of Dysmoder (Table 6, Fig. 4). The same phenomenon was observed, but in a more pronounced way, in Folsomia 15 16 manolachei, an ubiquitous species. At medium concentration the abundance of this species in Eumull 17 more than doubled under the influence of Dysmoder (Fig. 3, Tables 3 and 6). At high concentration its 18 abundance increased sixfold compared to Eumull alone. The abundance of Isotomiella minor nearly 19 doubled after lead application at low concentration (Table 6), then significantly decreased at high 20 concentration but its abundance was three times that observed in the absence of Dysmoder (Table 3). 21 The Lepidocyrtus lanuginosus population collapsed at high concentration of lead in the absence of Dysmoder, and its abundance remained unchanged below this theshold (Table 3). In the presence of 22 23 Dysmoder L. lanuginosus exhibited a significant increase in abundance when lead concentration 24 increased, reaching a maximum at medium concentration, then decreased at high concentration 25 (Table 6) but remained 38 times more abundant than in the absence of Dysmoder (Table 3). A small 26 although significant increase (around a tenth unit) in soil pH was observed at high concentration only 27 (Table 6), of the same order as when Eumull was alone (Table 3), and the presence of Dysmoder in a paired compartment did not influence the pH of Eumull. 28

In the absence of lead a few animals belonging to the Dysmoder group of species (*Micranurida pygmaea, Pseudosinella mauli, Sminthurinus signatus, Willemia anophthalma, Xenylla tullbergi*) were found in the Eumull compartment, and their number increased with lead concentration (Table 6, Fig. 1). Nevertheless this trend was not significant, due to strong departures from a box to another (see standard error values on Table 6).

6

7 The Dysmoder compartment was affected by the presence of the Eumull compartment. In the 8 absence of lead in the Eumull compartment, the number of ubiquitous species decreased significantly 9 in adjacent Dysmoder, as well as the density of the Dysmoder species Willemia anophthalma, and the 10 pH increased slightly but significantly (Table 7, Figs. 1 and 2). When lead was applied to Eumull, the 11 number and the abundance of Eumull species increased with lead concentration in the Dysmoder 12 compartment, their abundance reaching a level nearly twice that of unpolluted Eumull (Table 6). This 13 was mostly due to Parisotoma notabilis, the abundance of which at medium and high concentration of 14 lead reached in Dysmoder a level 2.8 and 2.5 times that of original Eumull (Fig. 4, Table 7). Other species increased their abundance in Dysmoder when lead was applied to Eumull, as for instance the 15 16 ubiquitous Isotomiella minor, the abundance of which trebled when lead was at medium concentration, and the ubiquitous Lepidocyrtus lanuginosus, the abundance of which near trebled at high 17 18 concentration (Table 7). The pH of Dysmoder increased with lead concentration (Table 7).

19

#### 20 4. Discussion

21

Despite the absence of significant colonization of the polluted neutral soil by strongly 22 acidophilic species such as Willemia anophthalma, which was yet well-represented in the acid soil 23 24 used for the experiment, migration movements were clearly demonstrated between the polluted mull 25 and the moder. In the presence of lead, moder acted as a refuge for the isotomid Parisotoma notabilis, 26 coming from the polluted mull. This species is known to live both in acid and neutro-acidocline soils 27 (Ponge 1993), although it proved to be highly sensitive to experimental acidification with sulphuric acid (Bååth et al., 1980; Hågvar and Kjøndal, 1981; Hågvar, 1984), preferred alkalinity in pH-preference 28 tests (Van Straalen and Verhoef, 1997) and was favoured by ash or lime application (Abrahamsen et 29 30 al., 1980; Hågvar and Abrahamsen, 1980; Vilkamaa and Huhta, 1986). It should be noticed that *P.notabilis*, also known for its sensitivity to heavy metals (Tranvik et al., 1993; Bruus Pedersen et al., 1999), was strongly depressed in our experiments at medium concentration of lead (6,000 ppm).
Contrary to moder, unpolluted mull did not act as a refuge for animals escaping contamination by heavy metals. Several reasons could explain this unexpected phenomenon, such as too many predators or a lack of food resources and habitats, but our experiment was not designed to explore these factors.

7

8 The colonization of the polluted mull by the isotomid Folsomia manolachei coming from the 9 moder was clear. This species was present in both humus forms, but it was much more abundant in 10 the moder, which acted as a source for the polluted mull. At medium lead concentration and only in 11 the presence of moder the abundance of this species increased in polluted mull compartments to a 12 level so high that it cannot be explained by another cause than a colonization by specimens coming 13 from the moder. Although the appearance of typical acidophilic species in the polluted mull was not 14 significant, their presence in lead-polluted mull compartments, even at high concentration (60,000 ppm), indicated that they were able to colonize soils polluted by heavy metals, but were poorly 15 16 attracted to them. One possible reason for the lack of a more intense colonization of the polluted mull by the mycetophagous poduromorph Willemia anophthalma (Ponge, 1991; Ponge, 2000b) could be 17 18 the scarcity of fungi in the mull at neutral pH used for the experiments, and a subsequent lack of 19 attraction by fungal odour (Bengtsson et al., 1988; Sadaka-Laulan et al., 1998), while fungi where 20 abundant in the acidic moder, as ascertained by visual inspection. Another reason could be the lack of 21 motility of this species, which has particularly short legs compared to that of entomobryomorph (entomobryid and isotomid) species. Isotomid species such as Folsomia manolachei and Parisotoma 22 notabilis have a less specialized feeding habit, their diet being mainly composed of humified organic 23 24 matter (Ponge, 1991; Ponge, 2000b). They are also much more motile than poduromorphs, due to 25 possessing longer legs and functional furcula (Hopkin, 1997), and these two species are indifferent to 26 soil pH (Ponge, 1993). These attributes make them better able to move to other places when 27 environmental conditions become unfavourable or when more space or food are available somewhere 28 else.

1 If we compare populations of Folsomia manolachei from the acid and neutral soils used for our 2 experiments, it appears that mull specimens were more sensitive to lead application than moder ones. 3 The existence of populations tolerant or intolerant to heavy metals in Folsomia manolachei could be 4 compared to the case of Orchesella cincta where heritability of tolerance to heavy metals has been 5 demonstrated (Posthuma, 1990; Posthuma et al., 1992; Posthuma et al., 1993). In our experiments animals attracted to a soil contaminated by heavy metals did not come from a polluted site but from an 6 7 acidic coniferous forest soil. The importance of tolerant strains of Folsomia manolachei for the 8 possible reclamation of polluted sites should be stressed, given its wide occurrence in all types of soils 9 (Rusek, 1989; Mateos and Selga, 1991; Ponge, 1993). As in our experiments with lead acetate, this 10 species recolonized soil cores polluted with copper sulphate where it exhibited higher densities than in 11 the unpolluted control (Filser and Hölscher, 1997), and was attracted to copper sulphate solutions in 12 choice experiments (Filser et al., 2000). The same phenomenon was observed with soil polluted by 13 copper-containing fungicides (Filser et al., 2000). In a naturally lead-contaminated site Hågvar and 14 Abrahamsen (1990) classified Folsomia quadrioculata (a nearby species) as tolerant to lead, its abundance being not affected at concentrations as high as 5,000 ppm. 15

16

17 Changes in pH cannot explain the above mentioned movements of collembolan populations 18 from a humus type to another. Although soil pH was significantly affected by the application of lead 19 acetate, the rise observed at the end of the experiment in the neutral soil was very weak (at most 0.1 20 pH unit), thus it was not high enough to force some species to escape from polluted compartments. A 21 possible attraction of acidophilic species by a decrease of pH in the mull soil can be disregarded for the same reason. We cannot rule out possible toxic (or attractive) effects of the acetate moiety, but the 22 23 absence of acidification of the soil at the end of the experiment led us to conclude that such effects, if 24 any, were probably temporary. Nevertheless, acetate toxicity could explain at least partly, together 25 with an increase in osmotic pressure of the soil solution (Heungens and Van Daele, 1984), the 26 collapse in collembolan abundance and diversity we observed at high concentration. Lead, acetate 27 and osmotic pressure acted probably both directly and indirectly on soil collembolan communities. Indirect effects could be suspected to occur through changes in predatory pressure (Grelle et al., 28 29 2000) and quantity and quality of the microflora (Tranvik and Eijsackers, 1989; Hopkin, 1994) which 30 may have occurred during the two weeks of the experiment.

	1		

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1 6. Figure captions

#### 2

Fig. 1 Abundance of Collembola (mean and standard error of five replicates) in experimental
 compartments (1 dm<sup>3</sup> each). E = Eumull. D = Dysmoder. E0 = Eumull without lead. E1 =
 Eumull with 50 ppm lead. E2 = Eumull with 6,000 ppm lead. E3 = Eumull with 60,000 ppm
 lead. Opposite bars relate to adjacent compartments

- 7
- Fig. 2 Number of species of Collembola (mean and standard error of five replicates) in experimental
   compartments (1 dm<sup>3</sup> each). E = Eumull. D = Dysmoder. E0 = Eumull without lead. E1 =
   Eumull with 50 ppm lead. E2 = Eumull with 6,000 ppm lead. E3 = Eumull with 60,000 ppm
   lead. Opposite bars relate to adjacent compartments
- 12
- Fig. 3 Abundance of *Folsomia manolachei* (mean and standard error of five replicates) in experimental
   compartments (1 dm<sup>3</sup> each). E = Eumull. D = Dysmoder. E0 = Eumull without lead. E1 =
   Eumull with 50 ppm lead. E2 = Eumull with 6,000 ppm lead. E3 = Eumull with 60,000 ppm
   lead. Opposite bars relate to adjacent compartments
- 17

Fig. 4 Abundance of *Parisotoma notabilis* (mean and standard error of five replicates) in experimental
 compartments (1 dm<sup>3</sup> each). E = Eumull. D = Dysmoder. E0 = Eumull without lead. E1 =
 Eumull with 50 ppm lead. E2 = Eumull with 6,000 ppm lead. E3 = Eumull with 60,000 ppm
 lead. Opposite bars relate to adjacent compartments

	Eumull	Dysmoder	Ubiquitous	Dubious
Allacma fusca (Linné, 1758)	1	0	0	0
Arrhopalites caecus (Tullberg, 1871)	1	0	0	0
Arrhopalites sericus Gisin, 1947	0	1	0	0
Dicyrtoma fusca (Lucas, 1842)	0	0	1	0
Dicyrtomina minuta (Fabricius, 1783)	1	0	0	0
Folsomia manolachei Bagnall 1939	0	0	1	0
Friesea truncata Cassagnau, 1958	0	0	1	0
Heteromurus nitidus (Templeton, 1835)	1	0	0	0
Isotomiella minor (Schäffer, 1896)	0	0	1	0
Isotomurus palustris (Müller, 1776)	1	0	0	0
Kalaphorura burmeisteri (Lubbock, 1873)	1	0	0	0
Lepidocyrtus lanuginosus (Gmelin, 1788)	0	0	1	0
Megalothorax minimus (Willem, 1900)	0	0	1	0
Mesaphorura gr krausbaueri (Börner, 190	0	0	1	0
Mesaphorura gr sylvatica (Rusek, 1971)	1	0	0	0
Mesaphorura gr yosii (Rusek, 1967)	0	0	1	0
Micranurida pygmaea (Börner, 1901)	0	1	0	0
Monobella grassei (Denis, 1923)	1	0	0	0
Neanura muscorum (Templeton, 1835)	1	0	0	0
Onychiurus pseudogranulosus Gisin, 1951	1	0	0	0
Orchesella cincta (Linné, 1758)	1	0	0	0
Paratullbergia callipygos (Börner, 1902)	0	0	1	0
Parisotoma notabilis (Schäffer, 1896)	1	0	0	0
Proisotoma minima (Absolon, 1901)	0	1	0	0
Pseudachorutes parvulus Böener, 1901	1	0	0	0
Pseudosinella alba (Packard, 1873)	1	0	0	0
Pseudosinella mauli Stomp, 1972	0	1	0	0
Sminthurinus aureus (Lubbock, 1862)	1	0	0	0
Sminthurinus signatus (Krausbauer, 1898)	0	1	0	0
Sphaeridia pumilis (Krausbauer, 1898)	0	0	1	0
Stenaphorurella denisi (Bagnall, 1935)	1	0	0	0
Tomocerus minor (Lubbock, 1862)	0	0	1	0
Willemia anophthalma Börner, 1901	0	1	0	0
Willemia buddenbrocki Hüther, 1959	1	0	0	0
Xenylla grisea Axelson, 1900	1	0	0	0
Xenylla tullbergi Börner, 1903	0	1	0	0
Xenvilodes armatus (Axelson, 1903)	0	0	0	1

**Table 1.** List of collembolan species found in the experiment, 0: absence, 1: presence

**Table 2.** Mean abundance ( $\pm$ S.E.) of most frequent collembolanspecies and groups of species and water pH in Eumull andDysmoder at the end of the experiment. Means followed by acommon letter indicate groups which do not differ significantly. E =Eumull species, D = Dysmoder species, U = ubiquitous species

		Eumull	Dysmoder
U	Dicyrtoma fusca	1.8±0.6	0.6±0.2
U	Folsomia manolachei	18.5±1.3b	113.4±18.6a
U	Friesea truncata	0.5±0.3	0.6±0.1
Е	Heteromurus nitidus	1.3±0.3a	0b
U	Isotomiella minor	51.3±3.8a	25.7±3.4b
U	Lepidocyrtus lanuginosus	1.6±0.3b	4.8±0.6a
U	Megalothorax minimus	0.5±0.5b	8.9±0.6a
D	Micranurida pygmaea	0b	1.9±0.6a
U	Paratullbergia callipygos	0.6±0.3b	3.1±0.5a
Е	Parisotoma notabilis	8.7±1.3a	0b
D	Proisotoma minima	0b	0.4±0.1a
Е	Pseudosinella alba	2.0±0.4a	0b
D	Pseudosinella mauli	0b	0.7±0.3a
D	Sminthurinus signatus	0b	1.4±0.3a
U	Sphaeridia pumilis	2.1±0.7	2.9±0.5
D	Willemia anophthalma	0b	28.6±3.0a
D	Xenylla tullbergi	0b	0.3±0.1a
	Total abundance	90.4±5.5b	194.6±17.2a
	Abundance of Eumull species	13.3±1.6a	0b
	Abundance of Dysmoder spec	0b	33.3±2.5a
	Abundance of ubiquitous spec	77.1±4.4b	161.3±16.4a
	Total number of species	8.9±0.4b	12.0±0.3a
	Number of Eumull species	4.8±0.5a	0b
	Number of Dysmoder species	0b	5.2±0.4a
	Number of ubiquitous species	6.4±0.7b	9.6±0.5a
	Water pH	7.66±0.03a	4.29±0.01b

**Table 3.** Mean abundance (±S.E.) of most frequent collembolan species and groups of species and water pH in Eumull after application of lead acetate in the two compartments of experimental boxes, compared to control boxes without addition of lead. Means followed by a common letter indicate groups which do not differ significantly. E = Eumull species, U = ubiquitous species. E0/E0 = No Pb, E1/E1 = Pb at 50 ppm, E2/E2 = Pb at 6,000 ppm, E3/E3 = Pb at 60,000 ppm

		E0/E0	E1/E1	E2/E2	E3/E3
U	Dicyrtoma fusca	1.8±0.6	1.2 <del>±</del> 0.3	2.0±0.7	0.5±0.2
U	Folsomia manolachei	18.5±1.3a	13.3±1.8b	8.1±1.0c	0.9±0.7d
U	Friesea truncata	0.5±0.3ab	1.5±0.5a	1.6±0.4a	0b
Е	Heteromurus nitidus	1.3±0.3a	0.6±0.2ab	1.0±0.2ab	0.3±0.2b
U	Isotomiella minor	51.3±3.8a	53.7±10.6a	46.8±7.9a	0.7±0.7b
U	Lepidocyrtus lanuginosus	1.6±0.3a	1.3±0.4a	1.2±0.3a	0.2±0.1b
U	Megalothorax minimus	0.5±0.5	0.1±0.1	0	0
U	Paratullbergia callipygos	0.6±0.3bc	1.0±0.2b	1.9±0.4a	0c
Е	Parisotoma notabilis	8.7±1.3a	5.8±0.3a	2.3±0.3b	0.2±0.2c
Е	Pseudosinella alba	2.0±0.4a	1.3±0.3ab	0.6±0.3bc	0c
U	Sphaeridia pumilis	2.1±0.7a	1.3±0.5ab	0.3±0.3b	0b
	Total abundance	90.4±5.5a	86.3±14.5a	68.3±9.6a	2.8±1.5b
	Abundance of Eumull species	13.3±1.6a	12.7±1.3a	6.4±0.7b	0.5±0.2c
	Abundance of ubiquitous spec	77.1±4.4a	73.6±13.7a	61.9±9.0a	2.3±1.3b
	Total number of species	8.9±0.4a	10.3±0.9a	9.1±0.3a	1.5±0.5b
	Number of Eumull species	4.8±0.5a	6.4±0.7a	5.8±1.0a	0.6±0.2b
	Number of ubiquitous species	6.4±0.7a	6.8±0.7a	6.0±0.0a	2.0±0.6b
	Water pH	7.66±0.03b	7.63±0.03b	7.65±0.01b	7.8±0.02a

**Table 4.** Mean abundance ( $\pm$ S.E.) of most frequent collembolan species and groups of species and water pH in unpolluted Eumull after application of lead acetate in the adjacent Eumull compartment, compared to control boxes without addition of lead. Means followed by a common letter indicate groups which do not differ significantly. E = Eumull species, U = ubiquitous species. E0/E0 = No Pb, E0/E1 = Pb at 50 ppm, E0/E2 = Pb at 60,000 ppm

		E0/E0	E0/E1	E0/E2	E0/E3
U	Dicyrtoma fusca	1.8±0.6	1.4±0.4	1.8±0.4	0.8±0.6
U	Folsomia manolachei	18.5±1.3a	16.8±1.9ab	12.4±0.5bc	9.6±2.1c
U	Friesea truncata	0.5±0.3	1.0±0.8	0.8±0.4	0.8±0.4
Е	Heteromurus nitidus	1.3±0.3a	0.4±0.2b	0.6±0.2ab	0b
U	Isotomiella minor	51.3±3.8	59.2±10.5	51.2±10.2	33.2±7.7
U	Lepidocyrtus lanuginosus	1.6±0.3	1.4±0.5	1.6±0.4	1.4±0.7
U	Megalothorax minimus	0.5±0.5ab	1.0±0.3a	0b	0b
U	Paratullbergia callipygos	0.6±0.3	0.4±0.2	1.6±0.7	1.6±0.6
Е	Parisotoma notabilis	8.7±1.3	7.8±0.7	8.8±2.7	3.8±0.9
Е	Pseudosinella alba	2.0±0.4	1.8±0.6	1.4±0.5	0.6±0.4
U	Sphaeridia pumilis	2.1±0.7	1.4±0.5	1.6±0.9	0.8±0.2
	Total abundance	90.4±5.5	94.0±13.8	84.6 <del>±</del> 8.9	55.2±8.5
	Abundance of Eumull species	13.3±1.6	11.2 <del>±</del> 0.9	13.6 <del>±</del> 2.7	6.8±2.2
	Abundance of ubiquitous spec	77.1±4.4	82.8±13.1	71.0±9.7	48.4±8.6
	Total number of species	8.9±0.4	9.4±0.9	10.8±0.7	8.6±0.9
	Number of Eumull species	4.8±0.5	3.2±0.5	4.8±0.6	3.2±0.9
	Number of ubiquitous species	6.4±0.7	6.2±0.6	6.0±0.4	5.4±0.6
	Water pH	7.66±0.03	7.68±0.01	7.66±0.02	7.78±0.02

**Table 5.** Mean abundance (±S.E.) of most frequent collembolan species and groups of species and water pH in lead acetate-polluted Eumull adjacent to unpolluted Eumull, compared to control boxes without any addition of lead in both compartments. Means followed by a common letter indicate groups which do not differ significantly. E = Eumull species, U = ubiquitous species. E0/E0 = No Pb, E1/E0 = Pb at 50 ppm, E2/E0 = Pb at 6,000 ppm, E3/E0 = Pb at 60,000 ppm

		E0/E0	E1/E0	E2/E0	E3/E0
U	Dicyrtoma fusca	1.8±0.6	1.4±0.4	1.6±0.4	0.8±0.6
U	Folsomia manolachei	18.5±1.3a	14.6±0.7a	14.8±2.0a	1.2±0.7b
U	Friesea truncata	0.5±0.3b	0.8±0.5b	2.0±0.4a	0.2±0.2b
Е	Heteromurus nitidus	1.3±0.3	0.6±0.4	0.6±0.2	0.2±0.2
U	Isotomiella minor	51.3±3.8a	52.6±8.4a	50.0±13.6a	1.4±0.7b
U	Lepidocyrtus lanuginosus	1.6±0.3	1.6±0.7	2.6±1.2	1.8±0.7
U	Megalothorax minimus	0.5±0.5	0.8±0.5	0.4±0.4	0
U	Paratullbergia callipygos	0.6±0.3	0.8±0.4	0.8±0.4	0
Е	Parisotoma notabilis	8.7±1.3a	6.0±0.6b	5.2±1.0b	0.4±0.4c
Е	Pseudosinella alba	2.0±0.4a	1.6±0.4ab	1.0±0.5ab	0.2±0.2b
U	Sphaeridia pumilis	2.1±0.7	1.6±0.9	1.4±0.7	0.4±0.2
	Total abundance	90.4±5.5a	84.0±9.7a	85.8±15.2a	7.8±2.0b
	Abundance of Eumull species	13.3±1.6a	9.6±1.0a	12.2±2.6a	2.0±0.7b
	Abundance of ubiquitous spec	77.1±4.4a	74.4±9.1a	73.6±14.0a	5.8±2.0b
	Total number of species	8.9±0.4a	9.0±0.7a	10.8±1.0a	4.8±0.7b
	Number of Eumull species	4.8±0.5a	3.4±0.7ab	4.6±0.9a	1.6±0.5b
	Number of ubiquitous species	6.4±0.7a	5.8±0.4a	6.2±0.7a	3.0±0.8b
	Water pH	7.66±0.03	7.67±0.01	7.73±0.04	7.73±0.05

**Table 6.** Mean abundance ( $\pm$ S.E.) of most frequent collembolan species and groups of species and water pH in Eumull adjacent to Dysmoder, at different levels of lead application in Eumull, compared to control boxes with unpolluted Eumull in both compartments. Means followed by a common letter indicate groups which do not differ significantly. E = Eumull species, D = Dysmoder species, U = ubiquitous species. E0/E0 and E0/D = No Pb, E1/D = Pb at 50 ppm, E2/D = Pb at 6,000 ppm, E3/D = Pb at 60,000 ppm

		E0/E0	E0/D	E1/D	E2/D	E3/D
U	Dicyrtoma fusca	1.8±0.6	1.6±0.7	1.4±0.5	1.0±0.6	0.8±0.4
U	Folsomia manolachei	18.5±1.3a	12.4±1.5a	12.2±1.7a	18.8±3.9a	5.6±0.6b
U	Friesea truncata	0.5±0.3ab	0.4±0.2ab	1.4±0.5ab	2.0±0.7a	0b
Е	Heteromurus nitidus	1.3 <del>±</del> 0.3	0.6±0.4	0.2±0.2	0.4±0.2	0.2±0.2
U	Isotomiella minor	51.3±3.8ab	38.4±6.4b	69.0±10.8a	57.6±6.6ab	2.0±0.4c
U	Lepidocyrtus lanuginosus	1.6±0.3b	2.2±0.4b	3.2±1.0ab	7.6±2.3a	3.6±0.4a
U	Megalothorax minimus	0.5±0.5ab	0b	1.2±0.4a	0.4±0.2ab	0b
D	Micranurida pygmaea	0	0	0	0.2±0.2	0
U	Paratullbergia callipygos	0.6±0.3ab	2.4±1.0a	0.6±0.4ab	0.6±0.2ab	0b
Е	Parisotoma notabilis	8.7±1.3a	6.2±1.6ab	6.2±2.2ab	3.0±0.8b	1.6±0.7b
Е	Pseudosinella alba	2.0±0.4a	0.2±0.2b	0.4±0.2b	1.2±0.4ab	0.2±0.2b
D	Pseudosinella mauli	0	0.2±0.2	0.2±0.2	0	0
D	Sminthurinus signatus	0	0.2±0.2	0	0	0
U	Sphaeridia pumilis	2.1±0.7	1.6 <del>±</del> 0.4	0.6±0.2	0.8±0.6	0.8±0.2
D	Willemia anophthalma	0	0	0.2±0.2	0.6±0.6	1.6±0.7
D	Xenylla tullbergi	0	0	0.2±0.2	1.6±1.6	0.2±0.2
	Total abundance	90.4±5.5ab	70.0±6.3b	99.6±11.2a	97.8±6.1a	17.4±2.6c
	Abundance of Eumull species	13.3±1.6a	10.4±1.1a	9.2±3.0a	5.8±0.7a	2.4±1.0b
	Abundance of Dysmoder spec	0	0.4±0.4	0.6±0.4	2.4±1.7	2.0±0.9
	Abundance of ubiquitous spec	; 77.1±4.4a	59.2±5.7b	89.8±10.8a	89.0±6.6a	13.0±1.2c
	Total number of species	8.9 <u>+</u> 0.4	10.8±1.0	9.8±0.9	9.6±1.1	7.2±1.0
	Number of Eumull species	4.8±0.5a	4.2±0.7a	2.8±0.6ab	3.2±0.5ab	1.6±0.5b
	Number of Dysmoder species	0	0.4±0.4	0.6±0.4	0.6±0.4	1.0±0.5
	Number of ubiquitous species	6.4±0.7	6.2 <del>±</del> 0.5	6.4±0.7	5.8±0.8	4.6±0.4
	Water pH	7.66±0.03b	7.64±0.03b	7.61±0.01b	7.68±0.02b	7.78±0.05a

**Table 7.** Mean abundance ( $\pm$ S.E.) of most frequent Collembolan species and groups of species and water pH in Dysmoder adjacent to Eumull, at different levels of lead application in Eumull, compared to control boxes with Dysmoder in both compartments. Means followed by a common letter indicate groups which do not differ significantly. E = Eumull species, D = Dysmoder species, U = ubiquitous species. E0/E0 and E0/D = No Pb, E1/D = Pb at 50 ppm, E2/D = Pb at 6,000 ppm, E3/D = Pb at 60,000 ppm

		D/D	D/E0	D/E1	D/E2	D/E3
U	Dicyrtoma fusca	0.6±0.2	1.2±0.5	0	1.2±0.4	1.0±0.0
U	Folsomia manolachei	113.4±18.6ab	152.2±12.0ab	182.6±23.6a	105.6±6.4b	116.6±23.2ab
U	Friesea truncata	0.6±0.1	1.2±0.6	1.4±0.4	0.4±0.4	1.8±0.5
Е	Heteromurus nitidus	0	0	0	0	0.2±0.2
U	Isotomiella minor	25.7±3.4b	29.4±3.2b	37.4±6.2b	84.6±7.7a	43.0±11.6b
U	Lepidocyrtus lanuginosus	4.8±0.6b	5.4±1.3b	7.4±1.2b	4.4±0.9b	13.2±1.1a
U	Megalothorax minimus	8.9±0.6b	8.6±0.8b	9.6±0.8b	12.4±1.4a	4.6±0.5c
D	Micranurida pygmaea	1.9±0.6	2.2 <del>±</del> 0.5	1.4±0.7	1.2 <del>±</del> 0.8	0.6±0.4
U	Paratullbergia callipygos	3.1±0.5a	2.6±0.9ab	0.4±0.2c	2.2±0.7ab	0.8±0.2bc
Е	Parisotoma notabilis	0c	1.0±0.4c	10.6±3.6b	24.4±4.5a	22.0±5.1a
D	Proisotoma minima	0.4±0.1b	3.0±1.1ab	6.0±0.6a	5.0±1.4ab	6.4±1.9a
D	Pseudosinella mauli	0.7±0.3	0.8±0.4	0.8±0.5	0.4±0.2	0.4±0.2
D	Sminthurinus signatus	1.4±0.3	1.0±0.4	1.0±0.3	0.6 <del>±</del> 0.2	1.8±0.6
U	Sphaeridia pumilis	2.9±0.5	2.6±1.1	1.6±0.7	0.6±0.4	0.8±0.2
D	Willemia anophthalma	28.6±3.0a	18.8±2.6bc	20.8±5.9abc	10.8±2.5c	17.4±0.9b
D	Xenylla tullbergi	0.3±0.1c	4.6 <del>±</del> 2.2	1.6±1.1	8.4±5.7	1.8±1.0
	Total abundance	194.6±17.2b	235.8±11.0ab	284.8±22.0a	264.4±12.2a	235.8±43.6ab
	Abundance of Eumull species	0c	1.2±0.5c	11.2±3.7b	24.6±4.6a	24.6±6.2a
	Abundance of Dysmoder spec	33.3±2.5	30.4±4.0	32.2±7.1	26.4±6.6	28.6±3.8
	Abundance of ubiquitous spec	161.3±16.4	204.2±11.3	241.4±21.1	213.4±13.4	182.6±35.5
	Total number of species	12.0 <del>±</del> 0.3	13.0±0.7	12.8±0.7	12.6±1.1	15.0±1.4
	Number of Eumull species	0c	0.8±0.4bc	1.4±0.2b	1.2±0.2b	2.4±0.5a
	Number of Dysmoder species	5.2±0.4	4.8±0.5	4.8±0.5	4.0±0.4	4.6±0.6
	Number of ubiquitous species	9.6±0.5a	7.2±0.6b	6.6±0.5b	7.4±0.7b	8.0±0.5ab
	Water pH	4.29±0.01d	4.33±0.02c	4.51±0.07abc	4.46±0.02b	4.65±0.03a



2 Fig. 1





2 Fig. 2





2 Fig. 3





2 Fig. 4