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Title: ACID-TOLERANT COLLEMBOLA CANNOT COLONIZE METAL-POLLUTED SOILS AT NEUTRAL PH

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Acid-tolerant Collembola cannot colonize metal-polluted soils at neutral pH

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Abstract

A microcosm experiment was performed to test the hypothesis that Collembola living in an acid soil (pH 4) were able to colonize a heavy metal-polluted soil of pH 7. After 6-month incubation, the added fauna were not recovered, except for a few individuals, while the original fauna were still as abundant as at the beginning of the experiment. It was concluded that, despite similarities between the chemical environment of acid soils and that of metal-polluted soils, differences in biotic and abiotic factors prevent acid-tolerant populations to survive and reproduce in a polluted site.

Keywords: Heavy metals, Collembola, Acid-tolerance, Inoculation

1. Introduction

The use of acid-tolerant organisms for the bioremediation of polluted soils has been proposed, on the basis of affinities between the chemical environment of acid and polluted soils, in particular those polluted with heavy metals (Chauvat and Ponge, 2002; Gillet and Ponge, submitted). The lack of metal specificity in detoxication mechanisms of soil invertebrates (Vandenbulcke et al., 1998; Köhler, 2002), and the presence of free (ionic) forms of aluminum, iron, manganese and other metals at low
pH (Tan, 1982), suggested that tolerance to acidity could allow tolerance to a broad range of heavy metals, too. However, neutral pH, excess of calcium and dramatic changes in food resources and habitat could affect survival and reproductive rate upon inoculation to metal-polluted sites (Kreutzer, 1995; Gillet and Ponge, 2002, 2003). The objective of this study was to initiate a self-reinforcing process by adding more, better adapted fauna, in order to i) stimulate humification (Bernier, 1998; Ponge, 1999; Davidson et al., 2002), ii) increase the number of micro-sites able to fix heavy metals (Senesi et al., 1987; Dupuy and Douay, 2001), thus preventing metals from circulating through the ecosystem.

Several experiments were performed to check whether acid-tolerant Collembola were able to colonize heavy-metal polluted soils. Short-term microcosm experiments using compartmented boxes showed that some species dispersed from an acid source (a dysmoder humus of pH 4) to a neutral soil heavily polluted with metals such as lead, zinc, or cadmium (Chauvat and Ponge, 2002; Gillet and Ponge, submitted). However, these dispersal experiments were of a too short duration for allowing reproduction and survival success to be assessed. A field site polluted with zinc, cadmium and lead was inoculated with microarthropods but resampling of the site after 7 and 14 months failed to reveal any significant dispersal from the inoculum, which kept its original population even after 14 months (Gillet, unpublished data). We suspected that the animals did not disperse to a great extent because they were not forced to leave their original habitat under environmental pressure (Tranvik and Eijsackers, 1989). As a consequence, it was decided to perform laboratory experiments, into which a known amount of acid-tolerant fauna could be introduced and monitored over six months, thus allowing the assessment of survival and reproduction of the introduced population. This time lapse was within a range between one generation time (1 to 2 months) and maximum longevity (1 to 2 years) for most Collembola (Siepel, 1994).
2. Materials and methods

The acid soil used for inoculating acid-tolerant fauna was a dysmoder humus (Bréthes et al., 1995) of pH H₂O 4.4 and pH KCl 3.3, originating from a beech forest at Willerzie, Belgium (Ponge et al., 1997; Gillet and Ponge, submitted). The metal-polluted soil was obtained from a field downwind a zinc smelter at Auby, France. It corresponded to the most polluted plot P1, as described in Gillet and Ponge (2002, 2003). The top 10 cm of the soil, which was a mor humus (Ponge et al., 2000) of pH H₂O 6.9 and pH KCl 6.5, contained 35,000 mg/kg of Zn (Gillet and Ponge, 2003), half of which was in a bioavailable form (Gillet and Ponge, submitted). The two soils were collected on the same day (2002.09.22). They included only ectorganic horizons, which were homogenized by hand in a large plastic sheet after discarding fresh litter and ground vegetation. After homogenization, samples were directly put in microcosms before transport to the laboratory.

Experimental microcosms were 11 x 8 x 4 cm (L x l x h) polystyrene boxes with lids, which were filled with soil, leaving enough overhead space for free movement of surface-active invertebrates. Lids were pierced at their centre with a 2 cm diameter hole, covered with nylon gauze, to allow gas exchange with the surrounding atmosphere. Ten replicates with the polluted soil (300 cm³) were kept closed without any further treatment. Ten other replicates were placed under Berlese funnels used for the extraction of microarthropods (Edwards and Fletcher, 1971) and were inoculated with fauna from 300 cm³ dysmoder humus. In parallel, microarthropods from 300 cm³ of both substrates were extracted using the same method. During the extraction, which took 10 days, uninoculated boxes were also incubated in the extractor. Once the inoculation was completed, all microcosms (10 inoculated, 10 uninoculated) were incubated in an air-forced chamber at constant temperature (15°C), in darkness.
Incubation lasted for 6 months, after which time microarthropods were extracted in the same way.

The microcosms were kept at field moisture, their weight being kept constant by adding deionized water each fortnight.

Extracted animals were collected and preserved in 95% ethyl alcohol. They were sorted under a dissecting microscope and mounted in chloral-lactophenol for examination in phase contrast microscopy. Several keys were used for the identification of Collembolan species. In particular the (still experimental) key by Hopkin (in prep.) was judged very handy. It was completed by more detailed monographs by Gisin (1960), Zimdars & Dunger (1994), Jordana et al. (1997), Fjellberg (1998), Bretfeld (1999) and Potapov (2001).

A special attention was apid to the tullbergiid species *Mesaphorura macrochaeta* Rusek. The sex ratio of adult specimens was determined after pooling all animals from the same treatment (Dysmoder, Original Mor, Incubated Mor, Inoculated-incubated Mor). We have noted before that this parthenogenetic species shifted to sexual reproduction under environmental stress, including pollution by heavy metals (Niklasson et al., 2000; Gillet and Ponge, 2003). The gut contents of all specimens of *M. macrochaeta* were examined under phase contrast and classified into 6 categories (Empty guts, Holorganic humus, Hemorganic humus, Fungi, Bacteria, Exuviae), since it has been shown that the same species changed its food diet when submitted to a higher dose of heavy metals (Gillet and Ponge, 2003). Gut contents were characterized for each individual. Most intestines were void or displayed only one food category. In cases where several categories were present in the same gut, each category was scored to the nearest 0.1, the sum of the scores being fixed to 1 for each individual.
Densities of the different species, as well as total abundance of Collembola and number of species per sample were compared between treatments using Mann-Whitney procedure at .05 significance threshold (Glantz, 1997).

3. Results

The species composition of the dysmoder humus largely differed from that of the polluted soil (Table 1, Fig. 1). The dysmoder humus exhibited more than ten times the total abundance of the mor (polluted) humus, and more than three times its number of species. Some species were present in the acid soil but were totally lacking in the neutral (polluted) soil, the most abundant ones being Protaphorura eichhorni (Gisin), Folsomia quadrioculata (Tullberg), Friesea truncata Cassagnau, Isotomiella minor (Schäffer), Mesaphorura tenuisensillata Rusek and Ceratophysella denticulata (Bagnall). They were classified as dysmoder species. Some others were present in the neutral soil, but were totally lacking in the acid soil, the most abundant ones being Protaphorura armata (Tullberg) and Lepidocyrtus cyaneus Tullberg. They were classified as mor species. Finally, some species were present in both substrates, such as M. macrochaeta, Parisotoma notabilis (Schäffer) and Sphaeridia pumilis (Krausbauer). They were classified as common species.

No males were recorded in M. macrochaeta from the acid soil (337 ind.), while a quarter of the adults were males in the population from the neutral soil (189 ind.). Prominent differences were observed between dysmoder and mor in the distribution of gut contents (Fig. 2). Holorganic humus was dominant in M. macrochaeta from the beech forest, while empty guts, followed by hemorganic humus, were dominant in the polluted soil before incubation. Fungi were also notable in acid soil guts. On the contrary fungi, together with bacteria and exuviae, were scarcely observed in the neutral, polluted soil.
After incubation, no decrease was observed in the number of individuals living in the polluted soil (Table 1), but the number of species decreased to a large extent (Table 1, Fig. 1). The collapse in species richness was due to the disappearance of epigeic species such as *Isotoma viridis* Bourlet, *L. cyaneus*, *Sminthurinus elegans* (Fitch), *S. pumilis* and *Willowsia nigromaculata* (Lubbock). However, two hemiedaphic species were also observed to disappear at the end of the incubation period, namely *P. notabilis* and *Micranurida pygmaea* Boerner. The dominant species *M. macrochaeta* and *P. armata*, which comprised 80% of the total population, were not affected at all by incubation conditions (Table 1).

Food habits of *M. macrochaeta* were not affected to a great extent by incubation, apart from a small increase in the proportion of empty guts (Fig. 2). After 6 months, hemorganic humus was still dominant in the food bolus, although mineral matter was practically absent in the holorganic humus used for the experiment, as ascertained by the composition of humus profiles (Gillet and Ponge, 2002). The sex ratio of *M. macrochaeta* changed little during the experiment, the proportion of males passing from a quarter to a third of the adult population (Table 1).

Inoculation with dysmoder fauna doubled the number of species of mor humus when compared with the incubated, uninoculated substrate (Table 1, Fig. 1), but it did not increase the number of individuals (Table 1). Thus, most introduced animals were not recovered at the end of the experiment. Only four dysmoder species were found after incubation, namely *F. truncata*, *P. eichhorni*, *Pseudosinella alba* (Packard) and *Pseudosinella mauli* Stomp, but only in very low densities (less than one specimen per microcosm in average). The abundance of *M. macrochaeta*, which was dominant in mor humus but was also present in dysmoder, was not affected by the inoculation procedure (Table 1), thus it can be concluded at first sight that most introduced
specimens of *M. macrochaeta* died during incubation. On the contrary, the other
dominant species in the polluted soil, *P. armata*, decreased in abundance when acid-
tolerant fauna was inoculated, while it did not in the absence of inoculation (Table 1).

The examination of gut contents of *M. macrochaeta* at the end of the incubation
period (Fig. 2) showed that inoculation of dysmoder fauna caused a small increase in
empty guts, a strong decrease in the fraction of hemorganic humus and a strong
increase in holorganic humus and fungi. The sex ratio of *M. macrochaeta* was not
affected by inoculation (Table 1), suggesting that the population recovered at the end
of the experiment was the original population of the mor humus.

**Discussion**

From our experimental results, it can be concluded that most inoculated
specimens died within 6 months, despite an increase in species richness due to a trace
population and thus that most acid-tolerant microarthropods were unable to colonize
heavily polluted soil used for the experiment. Interestingly, introduced species like *M.
macrochaeta*, which were abundant in the mor (polluted, neutral) humus, but were also
abundant in the dysmoder (unpolluted, acidic) humus, died too. Only the original
population of the polluted site can be considered tolerant to the environmental
conditions (chemical, biotic) prevailing in mor humus. We ruled out the possibility of a
mixing of *M. macrochaeta* specimens from the two sites in inoculated microcosms at
the end of the experiment. Even though the examination of gut contents (more fungi,
more holorganic humus, compared to uninoculated microcosms) could lead us to this
conclusion, the absence of any change in the sex ratio clearly indicated that no
parthenogenetic females from the dysmoder were added to the original population of
the mor humus. The constant density of *M. macrochaeta* following inoculation indicated
that no juveniles were added, too. The observed changes in gut contents could be
caused by i) the fall of some debris which accompanied the escape by animals of the
drying substrate in Berlese funnels (although most of them were retrieved and sorted
out by hand after inoculation), ii) the use of cadavers of introduced specimens by the
original fauna of mor humus. The second cause is hardly probable, since arthropod
exoskeletons were absent from *M. macrochaeta* intestines in inoculated microcosms,
although some were observed in the absence of inoculation (Fig. 2).

The duration of the experiment was enough to allow reproduction to occur,
given the generation time (from egg to egg) of most Collembola (Siepel, 1994). In the
presence of predators, the absence of any significant change in the collembolan
population (apart from the disappearance of rare species, which is expected in
microcosms) was indicative of an equilibrium condition. The disappearance of most
introduced specimens could be due to saturation of the original population (Longstaff,
1976; Bernier and Ponge, 1998; Winkler and Kampichler, 2000) or to unfavourable
food and environmental conditions. The first cause can be ruled out, given the
abundance of food (decaying roots, fungi, humus) present in the sampled holorganic
profiles, as ascertained from direct observation and from quantitative analyses (Gillet
and Ponge, 2002). However, poor food quality could be suspected, since the food
habits of introduced specimens of *M. macrochaeta* clearly differed from that of
conspecific specimens living in the polluted soil. It is remarkable that mineral matter,
which was nearly absent in the sampled profiles (Gillet and Ponge, 2002), was
retrieved in high amount in the gut contents of *M. macrochaeta*, even after 6 months.
This species was thus able to search for fine mineral particles (supposed to have
detoxifying properties) in an environment in which these were nearly absent. It can be
hypothesized that the population from the beech forest, where holorganic humus was
the main food source but could be consumed without any danger, was unable to mix it
with mineral matter in the polluted mor humus. The litter of the polluted mor was partly
made of plant debris from hyper-accumulating plants such as *Arabidopsis halleri* (L.)
O'Kane & Al-Shehbaz, which accumulates zinc in its above-ground parts (Sarret et al., 2002). The inadaptation of added specimens of *M. macrochaeta* to toxic food or soil solution is a possible cause for the observed phenomenon. Ecotoxicological tests on the isotomid springtail *Folsomia candida* have shown that reproduction and growth of Collembola were affected by heavy metals, in particular Cd and Zn, at doses far under those found in our polluted soil (Sandifer and Hopkin, 1996; Smit et al., 1997; Crouau et al., 1999), thus toxic effects were not unexpected. However, it has been demonstrated that tolerance to heavy metals varies between populations of the same species and that contamination by trace elements may select better-adapted genotypes (Posthuma, 1990; Chenon et al., 2000). In the case of the two populations we compared, that of Auby, living in a soil with 35,000 mg/kg Zn, 190 mg/kg Cd and 6000 mg/kg Pb (Gillet and Ponge, 2003) can be considered strongly adapted to heavy metals, either by a better selection of its food (Fountain and Hopkin, 2001; Tranvik and Eijsackers, 1989; present results) or by physiological adaptation such as increased metal excretion rate or shortening of the juvenile period (Posthuma et al., 1992, 1993) or by shifting from parthenogenesis to sexual reproduction (Niklasson et al., 2000; Gillet and Ponge, 2003; present results). At last, a shock effect caused by a sharp increase in pH when animals living at pH 4 were introduced into a soil of pH 7 can be another possible cause for the failure of colonization (Crouau et al., 1999), even for species know to live over a wide range of pH conditions such as *M. macrochaeta* (Ponge, 1993).

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References


Gillet, S., Ponge, J.F., submitted. Are acid-tolerant collembolan communities able to colonise metal-polluted soil?


Figure captions

Fig. 1. Distribution of dysmoder, mor and common species in the four treatments.

DYSMODER = original acid (unpolluted) soil. MOR = original neutral (polluted)
soil. MOR+6 = polluted soil after 6-month incubation in microcosms.
MOR+DYSMODER = polluted soil after inoculation with dysmoder arthropods
and 6-month incubation in microcosms. Letters indicate significant differences
between total numbers of species per sample (n = 10) according to the four
treatments (Mann-Whitney U test). Bars are means and whiskers are standard
errors of the means.

Fig. 2. Distribution of gut contents of *Mesaphorura macrochaeta* according to the four
treatments (codes of treatments according to Figure 1)
Table 1  Population parameters of Collembola in the original substrates (dysmoder, mor) and after 6-month incubation in microcosms (means of 10 replicates followed by standard errors). Dysmoder species which were retrieved at the end of the experiment in mor humus are indicated in bold type. Letters in indices indicate significant differences between treatments (Mann-Whitney U test).

<table>
<thead>
<tr>
<th>Species</th>
<th>Dysmoder</th>
<th>Mor (original)</th>
<th>Mor (incubated)</th>
<th>Mor (incubated, and inoculated with fauna from dysmoder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allacma fusca</td>
<td>0.1±0.1</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Anarthropites spinosus</td>
<td>0.5±0.2a</td>
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<td>0b</td>
<td>0b</td>
</tr>
<tr>
<td>Ceratophysella denticulata</td>
<td>11±0.8a</td>
<td>0b</td>
<td>0b</td>
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<td>Friesea truncata</td>
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<td>0b</td>
<td>0.8±0.3b</td>
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<td>Lipothrix lubbocki</td>
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<td>18.8±6.5b</td>
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<td>20.6±7.2b</td>
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<td>8±4.3ab</td>
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<td>Willoeia nigromaculata</td>
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<td>Total Collembola</td>
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<td>28.2±7.5b</td>
<td>26.5±7.3b</td>
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<td>Number of species</td>
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<td>1.5±0.2a</td>
<td>3.3±0.5b</td>
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Fig. 1

![Bar graph showing number of species for different categories.](image_url)
Fig. 2