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RESEARCH ARTICLE

Assessment of toxicity of heavy metal-contaminated soils toward Collembola in the paddy fields supported by laboratory tests

Manping Liu[1] · Jie Xu[2] · Paul Henning Krogh[3] · Jing Song[4] · Longhua Wu[4] · Yongming Luo[5] · Xin Ke[2]

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Abstract

Effects on soil Collembola of Cu, Zn, Pb, and Cd pollution from Cu smelters over 40 years were investigated in paddy fields from an area of Eastern China. We compared the field effects to those observed in single-species laboratory tests employing the hemiedaphic collembolan *Folsomia candida* and the epedaphic *Sinella curviseta* obtained from laboratory cultures and exposed to field-collected polluted soil. The results indicated that different collembolan species responded differently to the pollution in the fields and could be divided into sensitive, indifferent, and tolerant types accordingly. The abundance of sensitive species decreased as the pollution increased, but this was not the same for indifferent and tolerant species. The dominant species changed from sensitive to tolerant species as the pollution increased. The reproduction of *F. candida* and *S. curviseta* was most sensitive to the contaminated soil compared to growth and survival; the sensitivity of the two species was similar. The growth was more sensitive than the survival for *F. candida* but not for *S. curviseta*. The growth and survival of *F. candida* were much more sensitive than those of *S. curviseta*. Sensitivity of field populations of *F. candida* (EC_{10} 31 [15–46]) and hemiedaphic species *Folsomia quadrioculata* (EC_{10} 52 [0.7–102]) were comparable with sensitivity of the reproduction of *F. candida* in the single-species tests (EC_{10} 21 [14–27]), suggesting that single-species test based on laboratory cultures and field soil could be used to link laboratory and field data and then reflect the field situation. *S. curviseta* could be used as an epedaphic species in single-species tests and *F. quadrioculata* as an indicator species for assessment of field effect.

Keywords Pollution · Cu/Zn/Pb/Cd · Springtail · Species composition · Sensitivity · Single-species test

Introduction

To ensure sustainable use of land for agriculture, heavy metal pollution of soil must be understood in terms of its long-term implications for soil functioning and diversity (Austruy et al. 2016). Chemical analyses are essential for the evaluation of soil pollution, but have drawbacks when used as the sole source of information for prediction of soil ecotoxicity (Crouau and Moia 2006). Specifically, they contribute only limited information regarding the overall toxicity of a polluted soil because that factor is controlled by interactions between pollutants and the soil matrix; accordingly, ecotoxicity tests are necessary to complete an environmental effects assessment (Ardestani et al. 2014). Collembola are known as ideal test animals for ecotoxicological research (ISO 2014; Nursita et al. 2005) and have been used in different experiments to investigate the effects of environmental contaminants such as pesticides, soil fumigants, acid rain, heavy metals, fertilizers, radiation, PAH, and PCBs on animals (Sørensen and Holmstrup 2005; Wiles and Krogh 1998). Collembolan abundance as an estimate of toxicity has been useful in studies of pesticides (Holland et al. 2000; Peveling et al. 1999), atmospheric pollution (Bressan and Paoletti 1997), PCPs (Salminen and Haimi 1998), and aerial metal deposition (Pedersen and van Gestel 2001).

Manping Liu and Jie Xu contributed equally to this study.

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✉ Xin Ke
xinke@sibs.ac.cn

[1] Natural History Research Center, Shanghai Natural History Museum, Shanghai Science and Technology Museum, Shanghai 200127, China

[2] Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

[3] Department of Bioscience, Aarhus University, Vejlsøvej 25, DK-8600 Silkeborg, Denmark

[4] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

[5] Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

The effects of pollutants on Collembola in the field have been the subject to a great deal of attention. Naeem et al. (1994) reported that a declining biodiversity was consistent with reduced ecosystem function. However, because functional diversity can be difficult to measure, species diversity is usually estimated instead (Bengtsson 1998). Most investigations of the effects of heavy metals on Collembola in soils that have been conducted to date are from Europe (Fiera 2009; Santorufo et al. 2012a; Santorufo et al. 2012b), while only a few have been conducted with Chinese soils. The use of in situ soils is more representative of field conditions in a particular area than that of many of the artificial soils, such as OECD standard soil (OECD 2004, 2009). Furthermore, Lock and Janssen (2001) have expressed the need to use aged or natural soils representative of the area under consideration because they are more indicative of field conditions. An approach using field soils is therefore more useful for risk assessment of polluted sites or for the assessment of soils before and after remediation (Fava et al. 2000; Juvonen et al. 2000; Kuznetsova 2009; van Gestel et al. 2001). Recently, soil collembolan-predatory mite food chain, ^{15}N-labeled litter addition method, and antioxidant enzyme activities of Collembola were used to assess heavy metal ecotoxicity and soil functional change (Zhu et al. 2016; Dai et al. 2018). It is necessary to establish an assessment system for ecotoxicity of heavy metal-contaminated soil suitable to China. The present study was designed to assess the toxicity of heavy metal-contaminated paddy field soils from the Zhejiang Province, China, on Collembola in the field and the laboratory.

To accomplish this, a field investigation of the effects of increasing levels of heavy metal contamination on the species diversity of Collembola was conducted in paddy fields. In addition, single-species tests were performed in the laboratory studying the effects of heavy metals in paddy field soils on the growth, survival, and reproduction of Collembola. The parthenogenetic and hemiedaphic *Folsomia candida* and the sexually reproducing and epedaphic *Sinella curviseta* representing different life forms were used for the single-species tests. These two collembolans were chosen in order to compare their response to long-term heavy metal field exposure and to prove if they could be used to predict population effects observed in the long-term polluted field when tested in the laboratory with field-collected polluted soil. Finally, our study was aimed at selecting new appropriate collembolan field indicators of heavy metal pollution.

Materials and methods

Field sampling

Heavy metal-contaminated soil was collected from an intensely industrialized area (29° 56′ 13″–21″ N, 119° 54′ 44″–55′ 56″ E) in Fuyang County, Zhejiang Province, China. The soil was collected from a typical Chinese agricultural paddy field containing 3.2% humus (1.8% organic carbon), a pH-H_2O of 7.23, a pH-$CaCl_2$ of 7.05, a density of 1.26 g cm^{-3} dry soil, and a total cation exchange capacity of 18.2 meq 100 g^{-1} soil.

Generally, more than 90% of soil Collembola inhabits the top 10 cm of soil (Bengtsson and Rundgren 1988). Thus, soil cores at a depth of 0–10 cm were considered to be sufficient to sample most of the Collembola. Soil samples were collected from ten study sites (S1–S10) with an area of 20 × 20 m^2 each, and they were 250 m apart from each other at increasing distance from smelters in the contaminated area (Fig. 1). Six replicate soil cores (diameter, 5 cm; depth, 10 cm) were collected from a quadrate (5 m × 5 m) aimed at collembolan sampling at the center of each site. The six soil cores were mixed and used for analysis of heavy metals, since the soil of each sampling area was considered homogeneous concerning soil physico-chemical properties and metal distribution because of the agricultural paddy field management and the small sampling area. Additional soil samples, ca. 1 kg for each site, were brought to the laboratory for toxicity testing. No uncontaminated "clean" control site could be found in the area because of the widespread industrial pollution, so the least polluted site, S10, was used as a control.

Extraction of Collembola and classification

The soil cores were placed into polythene bags for transport to the laboratory. The Collembola were extracted from the soil cores in Tullgren funnels with a 2-mm mesh into ethylene glycol for 6 days. During extraction, the temperature was increased from 20 to 50 °C at 5 °C per day. The extracted Collembola were stored in 70% alcohol until identification using the keys of Christiansen and Bellinger (Christiansen and Bellinger 1980–1981), Fjellberg (1998), Yin (2000), and Yosii (1977).

Chemical analysis

Soil samples were air-dried, thoroughly mixed, and passed through a 0.2-mm sieve; after which, they were stored in plastic bags prior to chemical analysis. The total heavy metal concentration in soil was determined by digestion with *aqua regia* (McGrath and Cunliffe 1985). The heavy metal concentrations in the digests were subsequently determined by flame atomic absorption spectrometry (AAS, Varian SpectrAA 220FS, USA. The detection limits were 0.01 mg/L for Cd, 0.03 mg/L for Cu, 0.008 mg/L for Zn, and 0.1 mg/L for Pb analyses). When the metal concentrations in the digests were below the detection limit for analysis by flame AAS, a graphite furnace AAS was used (Varian 220FS, 220Z, Palo Alto, CA, USA. The detection limits were 0.01 μg/L for Cd, 0.3 μg/L for Cu, 0.15 μg/L for Zn, and 0.28 μg/L for Pb

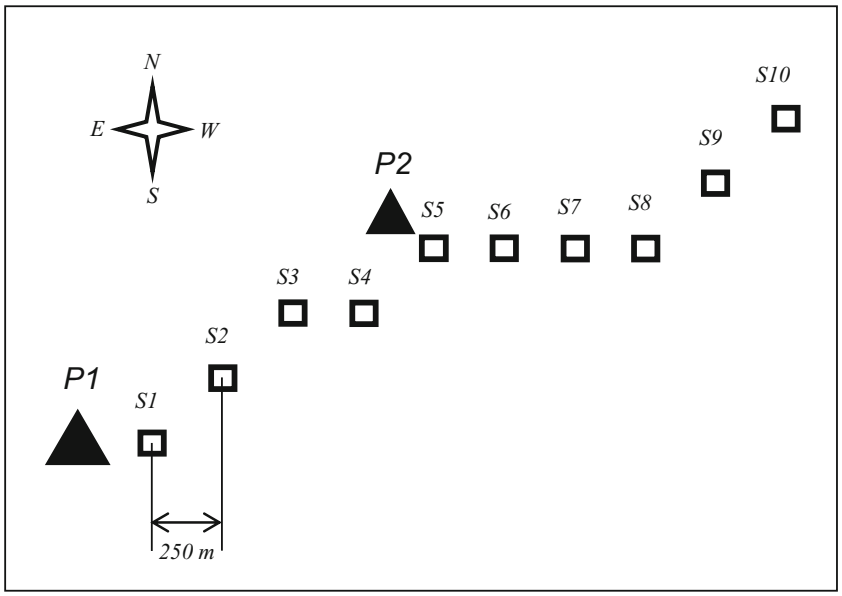

Fig. 1 Location of the ten study sites (S1–S10). P1 and P2, position of the two smelters

analyses). All glassware containers were soaked in 10% (*v*/*v*) HCl overnight and rinsed thoroughly with deionized distilled water before use. Data quality control consisted of the insertion of reagent blanks, duplicate samples, and reference materials into each batch of samples.

Animals for laboratory test

Cultures of *F. candida* (Berlin strain) were obtained from the soil fauna laboratory, University of Aarhus, Denmark, in 2000, and have since been kept permanently in culture in our laboratory. The initial *S. curviseta* population was collected from a botanical garden close to Shanghai and maintained in culture for several years in the laboratory. They were maintained in Petri dishes with a layer of moist plaster of Paris mixed with activated charcoal (9:1 *w*/*w*) at 20 ± 1 °C and 75% relative humidity (RH) with a 12:12-h dark:light (800 lx) regime and received distilled water and a small amount of dried baker's yeast (Angel Yeast Co., Ltd., Hubei, China) weekly. Synchronized *F. candida* were obtained according to standardized methods (OECD 2009) and *S. curviseta* according to the reproduction and synchronization methods (Xu et al. 2009a). The synchronized juveniles were used for experiments.

Exposure test in laboratory

The contaminated soils (S1–S10) from which the field soil fauna had been extracted were air-dried and passed through a 2-mm mesh. Thirty grams of soil was adjusted to a water content of 60% of the water holding capacity and placed into a plastic Sterilin pot (diameter, 6 cm; depth, 7 cm; total volume, 200 cm^3) with a plastic lid. The pots were then incubated at room temperature for 2 weeks to equilibrate the soil. After 2 weeks, the water content of the soil in the pots was checked and lost water was replenished with distilled water, and then ten synchronized animals were added to each pot and the lids were replaced. Four replicates were prepared for each of the ten soils, giving a total of 40 pots for each animal species.

The pots were maintained at the same conditions above for 28 days. During incubation, the lids were removed twice a week; 5 mg yeast food was added and some distilled water was sprayed inside of the lids. At the end of the test, the soil was extracted in Tullgren funnels, with the temperature increasing from 30 to 50 °C at a rate of 5 °C per day. Adults and juveniles were counted under a dissecting microscope. Digital photographs were taken (Olympus C-4000 zoom; image quality fine; macro-mode) and the length of the animals from the end of the posterior abdominal segment to the anterior margin of the head was measured by on-screen viewing by means of image analysis software (Photoshop 8.0).

Statistical analysis

Means of total collembolan abundance (*N*) and the species richness (*S*) were compared by one-way ANOVA (Statistica 7.0), with sampling sites as the factor. Multiple regression analysis was employed to reveal the relationship of *S* and *N* with total Cu concentrations. The results of Collembola exposure tests are presented as the average of four replicates. Between-site and replicate comparisons were made using ANOVA and Fisher's pairwise comparisons. Statistical analyses for estimation of LC and EC values for survival, reproduction, growth, and field population abundance were conducted using the NLMIXED procedure of SAS/STAT® version 9 (SAS Institute Inc., 2004). This procedure computes the approximate standard errors for the estimates using the delta method and then uses the standard errors to compute

corresponding 95% confidence limits (SAS Institute Inc., 2004). An exponential decay model or a linear model was used to estimate the relationship between a toxicity endpoint and the soil copper concentration. Mortality data were fit to a binomial distribution and linear link function.

Results

Field sampling

The *aqua regia*-extractable fractions of the heavy metals detected in the ten sampling sites are shown in Table 1. Four heavy metals were present in polluted soil, Cu at 12 to 1751 mg kg^{-1}, Zn at 136 to 3162, Pb at 44 to 1284, and Cd at 0.12 to 7.24. Comparison of the field concentrations of the four metals and their toxicities toward collembolans reported in previous studies (Fountain and Hopkin 2001; Hopkin and Spurgeon 2001; Lock and Janssen 2003) suggests that Cu and Zn are likely responsible for any ecotoxicological effects on springtails at these sites. The Cu concentrations were linearly correlated with those of Pb and Zn but not Cd in the present study, with the coefficients $R^2 = 0.03$ ($F_{(1,8)} = 0.25$, $P = 0.63$), $R^2 = 0.85$ ($F_{(1,8)} = 45.42$, $P < 0.001$), and $R^2 = 0.91$ ($F_{(1,8)} = 79.66$, $P < 0.001$) occurring for Cd, Pb, and Zn, respectively. Based on these findings, the Cu concentrations were considered to be representative of all total heavy metal concentrations measured in this study; thus, only correlations with Cu will be displayed for the remainder of the paper.

Species data

A total of 792 individuals belonging to 14 species of collembolans were obtained from the ten study sites (Table 2). The abundance of collembolans at the sites varied from 500 (± 96) individuals m^{-2} at site S1 to 71,166 (± 7332) individuals m^{-2} at site S7. Species with abundances greater than 10% of the total abundance were considered dominant and included *Tullbergia callipygos* (20.7%), *Isotoma* (*pseudisotoma*) *monochaeta* (17.4%), *Hypogastra matura* (18.6%), *Isotomodes fiscus* (18.6%), and *Folsomia quadrioculata* (13.5%).

The total number of collembolan species collected per site ranged from a minimum of 2 at site S1 and site S2 to a maximum of 10 at site S7 (Table 2). There were no species that were common to all the ten sites, and only three species were common to more than five sites, *Folsomia quadrioculata*, *Isotoma* (*pseudisotoma*) *monochaeta*, and *Tullbergia callipygos*. One-way ANOVA revealed significant differences in total collembolan abundances (N) ($F_{(9,50)} = 537.51$, $P < 0.01$) and species richness (S) ($F_{(9,50)} = 15.60$, $P < 0.01$) among the sites, with the maximum values of both being observed at site S7 and smaller values being observed for the control populations at S10. Multiple regression indicated a weak significant correlation between total Cu concentrations and S ($R^2 = 0.24$, $F_{(1,8)} = 2.3$, $P = 0.15$). However, no significant relationship was observed between total Cu concentrations and total collembolans ($R^2 = 0.12$, $F_{(1,8)} = 1.11$, $P = 0.20$) in any of the soil cores. Additionally, neither the value of S nor total Collembola were linearly correlated with total Cu concentrations.

Community structure analysis

According to Filser et al. (2000) collembolans are either sensitive or tolerant to soil contamination integrating both direct and indirect metal effects. We distinguished the following response types in contaminated areas: metal-sensitive (species extinct or severely reduced); indifferent (species showing no distinct response); and metal-tolerant (species maintaining or increasing their population size).

Based on the above classification criteria, we defined the species of response types in the contaminated areas (Table 3). Collembola species abundance was plotted against four ranges of Cu pollution according to environmental quality standard for soils in China (GB 15618-1995) (Table 3). Clean areas contained 0–35 mg Cu kg^{-1} dry soil (S9 and S10), while low-contamination areas contained 35–100 mg Cu kg^{-1} dry soil (S8 and S7), medium-contamination areas contained 100–400 mg Cu kg^{-1} dry soil (S4, S6, and S3), and high-contamination areas > 400 mg Cu kg^{-1} dry soil (S1, S2, and S5). The numbers of species at the four levels of contamination were 6, 12, 11, and 8, respectively, and the densities of collembolans were 15,333, 86,833, 23,833, and 6000 individuals/m^2, respectively.

The community structure analysis showed that both species richness (S) and collembolan abundance (N) changed in different ranges of Cu pollution (Table 3). Generally, as the Cu concentration increased, the sensitive species decreased

Table 1 Concentration of heavy metals in soil from the ten study sites (S1–S10)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cu (mg kg^{-1})	1751	605	348	167	816	287	75	47	22	12
Cd (mg kg^{-1})	1.30	7.00	7.24	1.50	1.76	1.49	0.76	0.37	0.21	0.12
Pb (mg kg^{-1})	1284	151	111	93	974	305	101	75	50	44
Zn (mg kg^{-1})	3162	1102	924	736	2445	1157	387	321	136	140

Table 2 Species richness and individual abundance of collembolans in the soil cores from the ten study sites (S1–S10) along with the Cu concentration gradient (the six replicate cores of each study site together)

Cu concentration (mg kg^{-1}) (study site)	12 (S10)	22 (S9)	47 (S8)	75 (S7)	167 (S4)	287 (S6)	348 (S3)	605 (S2)	816 (S5)	1751 (S1)
Species										
Arrhopalites caecus	2	1	–	–	–	–	–	–	–	–
Entomobrya griseoolivata	–	–	–	–	2	9	5	–	–	–
Folsomia candida	14	5	–	11	2	–	–	–	–	–
Folsomia diplophthalma	2	–	–	1	–	–	–	–	–	–
Folsomia quadrioulata	12	8	18	34	28	5	1	–	–	1
Hypogastra matura	–	–	24	120	–	2	–	1	–	–
Isotoma (*pseudisotoma*) *monochaeta*	–	–	11	49	34	21	19	–	4	–
Isotomodes fiscus	–	–	16	124	–	–	4	–	1	2
Neanura (*Lathriopyga*) *illines*	–	–	–	1	–	–	–	–	3	–
Sinella curviseta	2	–	–	1	2	–	1	–	–	–
Sminthuridae (*Sminthuridae*) *macnamarac*	–	–	–	2	2	1	–	4	–	–
Sphyrotheca multifasciata	–	–	1	–	–	3	–	–	–	–
Tetracacanthella arctica	–	–	10	–	–	1	–	–	1	–
Tullbergia callipygos	29	17	14	84	1	–	–	–	19	–
Total										
Species number	6	4	6	10	7	7	5	2	5	2
Individual number	61	31	94	427	71	42	30	5	28	3

significantly. Specifically, the tolerant species first increased in the lower range and then deceased in the two highest ranges, while the indifferent species also first increased but then did not change in the highest concentration ranges. As the Cu concentration increased from 0–35 to > 400 mg Cu kg^{-1}, the sensitive species decreased from two species to one species and then disappeared. The richness and abundance of tolerant species first increased and then decreased in the higher Cu concentration ranges. For indifferent species, these factors also initially increased, but these changes were not great and no further changes were observed at higher concentration ranges. In the range of 0–35 mg Cu kg^{-1}, *Tullbergia callipygos* (indifferent, 54.00%), *Folsomia quadrioulata* (indifferent, 20.00%), and *Folsomia candida* (sensitive, 18.99%) were dominant, comprising 92.99% of the species at this range. In the range of 35–100, *Hypogastra matura* (tolerant, 27.64%), *Isotomodes fiscus* (tolerant, 26.87%), *Tullbergia callipygos* (indifferent, 18.81%), and *Isotoma* (*pseudisotoma*) *monochaeta* (tolerant, 11.52%) were dominant, comprising 84.84% of the total. At 100–400, *Isotoma* (*pseudisotoma*) *monochaeta* (tolerant, 51.75%), *Folsomia quadrioulata* (indifferent, 23.78%), and *Entomobrya griseoolivata* (tolerant, 11.19%) were dominant, comprising 86.72% of the 11 species. At greater than 400, *Tullbergia callipygos* (indifferent, 52.78%), *Sminthurides macnamarai* (indifferent, 11.11%), and *Isotoma* (*pseudisotoma*) *monochaeta* (tolerant, 11.11%) were dominant, comprising 75% of the species.

Laboratory testing

After 4 weeks of exposure to the soil from the study sites, adult number of *F. candida* ($F_{(9,30)} = 12.32$, $P < 0.01$) and *S. curviseta* ($F_{(9,30)} = 5.28$, $P < 0.01$) subject to different Cu concentrations were significantly different (Fig. 2b). Specifically, the survival of *F. candida* at the two highest Cu concentrations of soil (1751 mg kg^{-1} (S1) and 816 mg kg^{-1} (S5)) was 50% lower ($P < 0.05$) than that of the control (12 mg kg^{-1} Cu (S10)). The number of adults (y) of *F. candida* ($y = -0.0025x + 8.9$, $R^2 = 0.86$) and *S. curviseta* ($y = -0.0014x + 9.53$, $R^2 = 0.66$) was significantly negatively correlated with the total Cu concentrations (x).

Reproduction (juveniles produced) of *F. candida* ($F_{(9,30)} = 52.35$, $P < 0.01$) and *S. curviseta* ($F_{(9,30)} = 66.29$, $P < 0.01$) also differed significantly among Cu concentrations (Fig. 2a). Specifically, there was no reproduction of *S. curviseta* and only three juvenile *F. candida* at the highest concentration. The number of juveniles produced (y) by *F. candida* ($y = -34.44 \ln(x) + 254.02$, $R^2 = 0.98$) and *S. curviseta* ($y = -14.47 \ln(x) + 103.87$, $R^2 = 0.94$) was significantly negatively correlated with log-transformed total Cu concentrations (x).

Body length of *F. candida* ($F_{(9,30)} = 28.8$, $P < 0.01$) and *S. curviseta* ($F_{(9,30)} = 2.94$, $P < 0.05$) differed significantly among Cu concentrations (Fig. 2c). Specifically, the length of *F. candida* varied from 1.20 mm at the highest

Table 3 Species of metal-sensitive, indifferent, and metal-tolerant collembolans found in the ten study sites and their densities (individuals m^{-2}). The results are the means (±standard deviation) of 12 replicates for Cu concentration in scope of < 35(S10, S9) and 35–100 (S8, S7) and 18 replicates for Cu concentration in scope of 100–400 (S4, S6, S3) and > 400 (S2, S5, S1)

Species	Cu concentration (mg kg^{-1} dry soil)							
	< 35		35–100		100–400		> 400	
Metal-sensitive								
Arrhopalites caecus	250 ± 131 a	C	0 b	R	0 b	R	0 b	R
Folsomia candida	1583 ± 529 a	D	917 ± 514 ab	C	111 ± 111 b	C	0 b	R
Metal-tolerant								
Entomobrya griseoolivata	0 b	R	0 b	R	889 ± 322 a	D	0 b	R
Hypogastra matura	0 b	R	12,000 ± 7996 a	D	111 ± 111 b	C	56 ± 56 b	C
Isotoma (*pseudisotoma*) *monochaeta*	0 b	R	5000 ± 2198 a	D	4111 ± 963 a	D	222 ± 173 b	D
Isotomodes fiscus	0 b	R	11,667 ± 5391 a	D	222 ± 173 b	C	167 ± 121 b	C
Indifferent								
Folsomia diplophthalma	167 ± 112 a	C	83 ± 83 a	R	0 a	R	0 a	R
Tetracacanthella arctica	0 a	R	833 ± 534 a	C	56 ± 56 a	R	56 ± 56 a	C
Sminthuridae (*Sminthuridae*) *macnamarac*	0 a	R	167 ± 112 a	R	167 ± 121 a	C	222 ± 101 a	D
Neanura (*Lathriopyga*) *illines*	0 a	R	83 ± 83 a	R	0 a	R	167 ± 167 a	C
Sinella curviseta	167 ± 167 a	C	83 ± 83 a	R	167 ± 121 a	C	0 a	R
Sphyrotheca multifasciata	0 a	R	0 a	R	167 ± 167 a	C	0 a	R
Tullbergia callipygos	4500 ± 1454 ab	D	8167 ± 4147 a	D	56 ± 56 b	R	1056 ± 488 b	D
Folsomia quadrioulata	1667 ± 689 ab	D	4333 ± 1163 a	C	1889 ± 836 ab	D	56 ± 56 b	C

Figures followed with identical small letters are not significantly different between Cu concentrations for each species (Turkey's test at 5% level of significance)

D dominant groups (> 10%), *C* commonly seen groups (1–10%), *R* rare groups (0.1–1%)

concentration to 1.79 mm at the lowest concentration, with an overall reduction of 33%. However, there were no differences in *S. curviseta* growth (body length) between any of the metal concentrations and the control. The length (y) of *F. candida* ($y = -0.13\ \ln(x) + 2.15$, $R^2 = 0.93$) and *S. curviseta* ($y = -0.025\ln(x) + 1.69$, $R^2 = 0.53$) was significantly negatively correlated with log-transformed total Cu concentrations (x).

Toxicity in the field and laboratory

For *F. candida* in the single-species tests, the EC_{10}, which is the concentration where the reproduction or growth is reduced by 10% in relation to the control, and the EC_{50}, the concentration with a 50% reduction of the reproduction or growth, were about 17 and 11 times lower than the LC_{10}, the concentration with an increase of 10% in mortality, and LC_{50}, the concentration with 50% mortality, respectively. The EC_{10} of growth was approximately five times less than the LC_{10} of survival, i.e., more sensitive, while three times higher than the EC_{10} for reproduction (Table 4). Comparing the results of the field and laboratory testing revealed that EC_{10} and EC_{50} values of the field population of *F. candida* were only slightly higher, 48 and 13%, respectively, than those of reproduction in the single-species tests. In the laboratory, the EC_{10} and EC_{50} of *S. curviseta* reproduction were approximately 25 and 21 times less than the LC_{10} and LC_{50} of survival, respectively. The EC_{10} for growth was 25 times higher than the EC_{10} of reproduction and slightly higher than the LC_{10} for survival (+ 3.6%). Comparison of the toxicity of *F. candida* and *S. curviseta* revealed that the EC_{10} and EC_{50} of reproduction of *F. candida* were approximately 24 and 14% lower than those of *S. curviseta*, respectively, while the LC_{10} and LC_{50} of survival of *F. candida* were both approximately half those of *S. curviseta*, and the EC_{10} of *F. candida* growth was much less than that of *S. curviseta* (approximately 12 times). Since *F. quadrioculata* was dominant and common at the field sites, the metal toxicity to *F. quadrioculata* in the field was also analyzed and compared with that of *F. candida*, and the results revealed higher EC_{10} and EC_{50} values for *F. quadrioculata* (approximately 68%).

Discussion

The results showed that different species of soil Collembola responded differently to pollution in long-term heavy metal-contaminated fields. Specifically, single-species tests using collembolans from laboratory cultures and field-collected

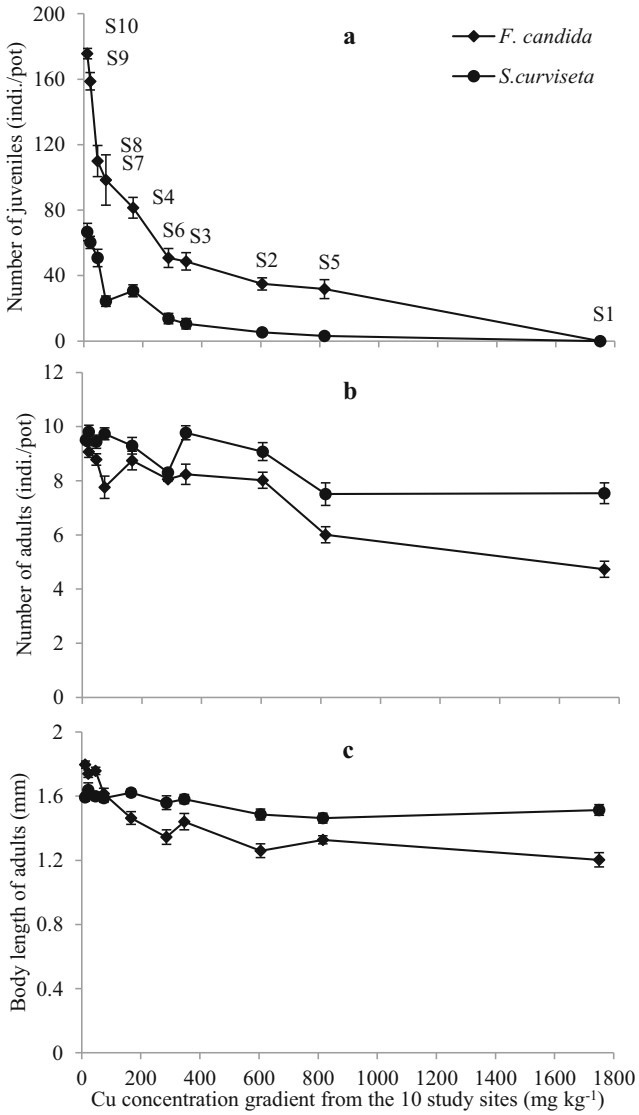

Fig. 2 Reproduction (**a**), survival (**b**), and growth (**c**) of *F. candida* and *S. curviseta* after 28 days of exposure in single-species test to the soils from the ten study sites

contaminated soils could be employed to predict the effects observed in the fields. Additionally, *S. curviseta* cultured in the laboratory was found to be a potential indicator species in single-species tests, while *F. quadrioculata* was a potential indicator species for field assessment.

Response of Collembola species composition to heavy metal-contaminated soils in fields

The Cu smelters were among the strongest sources of heavy metal pollution in this area. The Cu smelting caused the pollution of four metals Cu, Zn, Pb, and Cd. Since the four metals came from the same pollution sources of Cu smelting and therefore their concentrations were linearly correlated with each other though Cd was not, they were considered having similar concentration gradient trends along the study sites.

Table 4 Summary of heavy metal toxicity in terms of EC/LC estimates of laboratory and selected field effects in relation to soil copper concentration

Species	Endpoint		Cu (mg kg^{-1})	95% C.L.
F. candida	Survival	LC_{10}	355	[241–469]
	Reproduction	EC_{10}	21	[14–27]
	Growth	EC_{10}	68	[41–95]
	Field population	EC_{10}	31	[15–46]
	Survival	LC_{50}	1560	[1097–2023]
	Reproduction	EC_{50}	135	[91–180]
	Field population	EC_{50}	153	[73–232]
S. curviseta	Survival	LC_{10}	645	[334–956]
	Reproduction	EC_{10}	26	[21–32]
	Growth	EC_{10}	880	[459–1301]
	Survival	LC_{50}	3089	[1653–4525]
	Reproduction	EC_{50}	174	[140–207]
F. quadrioculata	Field population	EC_{10}	52	[0.7–102]
		EC_{50}	258	[3.6–512]

The four metals are usually toxic to animals; therefore, impacts of the contaminated soils on the collembolans were considered a joint effect of the four heavy metals, and the Cu concentrations were assumed to be representative for the four heavy metal concentrations. Since the sources were located at individual points, they created a gradient impact on surrounding ecosystems, which is convenient for studies of anthropogenic transformations of the environment (Kuznetsova 2009). Heavy metals can indirectly affect Collembola by damaging food resources, or directly through breathing valves (Fiera 2009); however, Collembola have certain preadaptations to pollution such as the ability to remove heavy metals with the exuvium and intestinal epithelium shed during molting (Joosse and Buker 1979). The metal distribution at the location was heterogeneous. For example, the Cu concentration in soil could range from 12 to 1751 mg kg^{-1} dry soil within a few hundred meters; therefore, the area was considered ideal for investigation of the effects of metals on Collembola within a small area of uniform climate and vegetation type. The collembolan densities observed in the present study were within the range observed in other studies of 10^3 to over 10^5 individuals m^{-2} in agricultural land (Russell and Alberti 1998), and the species abundance distribution was characterized by a high number of rare species and low number of dominant species, as in other studies (Chagnon et al. 2000).

The maximum population abundance and species richness were not observed at the control site, but instead in low-contamination areas. Furthermore, there was no significant relationship between total Cu concentration and the population abundance. Bengtsson and Rundgren (1988) reported that neither Collembola density nor diversity was linearly correlated with metal concentrations, but instead showed

a bell-shaped distribution. However, in the present study, multiple regression analysis showed that species richness was related to the total Cu concentration. Rusek and Marshall (2000) showed that the actual species present appeared to be a more reliable indicator of metal pollution than abundance and diversity, which is similar to the results reported in other studies (Bengtsson and Rundgren 1988; Bruce et al. 1999; Bruce et al. 1997; Russell and Alberti 1998; Smit and Van Gestel 1996). Taken together, these findings suggest that species richness is more sensitive than individual abundance.

Filser (1995) suggested using Collembola as an indicator of metal pollution because they express a range of species sensitivities. Tranvik et al. (1993) found that Collembola species-specific differences in heavy metal susceptibility resulted in decreasing abundance of certain species along pollution gradients, whereas other species maintained or even increased their populations. Similar results were found in the present study demonstrating that metal-sensitive species were severely or completely reduced as the Cu concentration increased from 0–35 to more than 400 mg Cu kg^{-1}, while the richness and abundance of indifferent and tolerant species were maintained. Additionally, the type of dominant species changed from more sensitive species to more tolerant or indifferent species, and the proportion of dominant species to the total abundance decreased from 92 to 75%. Similarly, other studies have shown that some tolerant species increased and sensitive species decreased under stress, such as low pH, high metal concentrations, and plowing, while other species maintained their populations at low levels in highly contaminated soils (Fiera 2009; Hågvar 1994; Haimi and SiiraPietikainen 1996).

The mechanisms behind the observed responses are poorly understood. Competition appears to be one of the most important factors influencing the collembolan community composition during chemical stress (Krogh 1991; Rusek and Marshall 2000). 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. A population can become extinct or decrease in number due to direct metal toxicity, but trophic interactions also play an important role. Some species may even become resistant to pollution and occur in higher numbers following exposure (Fiera 2009). Furthermore, if metals have a toxic effect that results in lower abundance of the main food source of a species, the consumer population density will decrease, while toxic effects on predators or competitors may result in increased populations (Filser et al. 2000). Accordingly, further studies to identify the exact mechanisms influencing the abundance of the 14 species of Collembola observed in the present study are warranted.

Response of population features of cultured Collembola species to the contaminated soils in laboratory

The adult survival and reproduction of both *F. candida* and *S. curviseta* and the growth of *F. candida* but not *S. curviseta* differed significantly among Cu concentrations in soils and were negatively correlated with the Cu concentrations, suggesting that both species were sensitive to contaminated soils from the field and that the single-species tests with the two species from laboratory cultures and the field-collected soils can be practically applied for the assessment of soil pollution. Although we were unable to determine which life stage of the collembolans were affected during this experiment (Xu et al. 2009b), the overall effect was a reduction in the population, which could be detrimental to the survival of a species at a contaminated site. In our previous investigation of *S. curviseta* with artificial soils (Xu et al. 2009a), the adult survival, reproduction, and growth were affected by soils amended with Cu, Zn, and Pb, respectively, which is consistent with the results of the present study. To the best of our knowledge, there is no literature regarding the tolerance of *S. curviseta* to heavy metals. The different sensitivity of growth to the contaminated soils between *S. curviseta* and *F. candida* may have occurred because *S. curviseta* is a surface-active edaphic species with higher mobility and less contact with soil pore water than the cuticle of *F. candida*. Russell and Alberti (1998) found that the numbers of euedaphic species at a clean site were higher than those at a metal-contaminated site, and that there were more hemiedaphic and epedaphic species at the contaminated site.

Toxicity of the contaminated soils to some Collembola species in the field and laboratory

The EC_{10} and EC_{50} of reproduction were much lower than the LC_{10} and LC_{50} of adult survival, respectively, for both *F. candida* and *S. curviseta*. For *F. candida*, the EC_{10} of growth was much less than the LC_{10} of survival, but much higher than the EC_{10} of reproduction, while for *S. curviseta*, the EC_{10} of growth was slightly higher than the LC_{10} of survival and much higher than the EC_{10} of reproduction. These findings suggest that reproduction is most sensitive to the contaminated soil compared to survival and growth, while growth is more sensitive than survival for the hemiedaphic species *F. candida*, but not the epedaphic *S. curviseta*. The LC_{10} and LC_{50} and EC_{10} for growth of *F. candida* were much lower than those of *S. curviseta*, which may also suggest that *F. candida* is more sensitive than *S. curviseta*. The EC_{10} and EC_{50} of the reproduction were similar between *F. candida* and *S. curviseta*, which was consistent with the finding that the reproduction of both was significantly negatively correlated with the total Cu concentrations during laboratory testing. However, the results observed in the field study differed

(Table 3). Specifically, the findings for *F. candida* in the field were consistent with those of laboratory testing, but those of *S. curviseta* were not. One possible reason for this is the different conditions between the field and the laboratory. Our previous study showed that *S. curviseta* preferred to oviposit on deadwood or decomposing leaves over soil (Xu et al. 2009a). This behavior might prevent eggs from being exposed to heavy metals in the fields. However, in the laboratory, the soils were air-dried and sieved through a 2-mm mesh, which made them homogeneous and destroyed their three-dimensional structure. Thus, *S. curviseta* was forced to oviposit in the soil pores, resulting in their reproduction being analogous to that of *F. candida*.

EC_{10} and EC_{50} of the field population of *F. candida* were only slightly higher than those of reproduction in a laboratory testing, suggesting that the results from the field and from laboratory testing are comparable.

The EC_{10} and EC_{50} of the *F. quadrioculata* population were slightly higher than those of *F. candida* in the field, suggesting that the sensitivity of *F. quadrioculata* is comparable to that of *F. candida*. *F. quadrioculata* is a dominant species in the field, so it is a potential indicator species.

Conclusions

In long-term heavy metal-contaminated fields, total soil Collembola are not linearly correlated with the metal concentrations because they consist of a range of species that differ in sensitivity to pollution. The species could be classified as sensitive, indifferent, and tolerant, and their performance should be considered when soil Collembola are used to assess soil contamination. The abundance of sensitive species could decrease or they can disappear completely in response to increasing pollutant concentrations, while the abundance of tolerant or indifferent species simultaneously increases. Accordingly, the presence or absence of sensitive and tolerant species in highly contaminated soils may serve as a good indicator of the soil condition. The results of single-species tests with edaphic collembolans from laboratory cultures and field-collected soils were comparable with those of field investigation, suggesting that the results of single-species tests in laboratory could reflect the soil pollution situation of the contaminated fields. *S. curviseta* could be used as epedaphic species in single-species test and *F. quadrioculata* has the potential for use as an indication species in field assessment.

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