# EFFECT OF LEACHING AND AGING ON THE BIOAVAILABILITY OF LEAD TO THE SPRINGTAIL *FOLSOMIA CANDIDA*

KOEN LOCK,*† NADIA WAEGENEERS,‡ ERIK SMOLDERS,‡ PEGGY CRIEL,† HILDE VAN EECKHOUT,† and COLIN R. JANSSEN†

†Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, J. Plateaustraat 22, 9000 Gent, Belgium
‡Katholieke Universiteit Leuven, Laboratory for Soil and Water Management, Kasteelpark Arenberg 20, 3001 Leuven, Belgium

**Abstract**—Because it is unclear if leaching can account for differences in metal bioavailability observed between metal-spiked soils and historically contaminated fi soils, we simultaneously assessed Pb toxicity to the springtail *Folsomia candida* in three transects of Pb-contaminated soils and in leached and unleached soils spiked at similar total Pb concentrations. Total Pb concentrations of 3,877 mg/kg dry weight and higher always caused signifi effects on *F. candida* reproduction in the spiked soils. In the transects, only the soil with the highest Pb concentration of 14,436 mg/kg dry weight signifi affected reproduction. When expressed as pore-water concentrations, reproduction was never signifi affected at Pb concentrations of 0.539 mg/L, whereas reproduction was always signifi affected at Pb concentrations of 0.678 mg/L and higher, independent of the soil treatment. These results indicate that pore-water Pb concentrations can explain, at least in part, the observed differences in the toxicity data expressed as total Pb concentrations. Leaching after the spiking procedure only caused small differences in Pb toxicity and, therefore, cannot account for toxicity differences between laboratory-spiked soils and historically contaminated fi soils.

**Keywords**—Lead toxicity Metal partitioning Percolation Soil-quality criteria Risk assessment

## INTRODUCTION

In laboratory toxicity tests assessing the effect of metals on soil organisms, metals usually are spiked in a single application as metal solutions. Little time is allowed for equilibration, and the associated counterions are not leached from the soil. Under fi conditions, however, metals often are not added as highly soluble salts, and even when this is the case, leaching through rainfall will continually remove the counterions from the topsoil during the accumulation period. The counterion can have a direct toxic effect or induce an indirect effect by raising the salinity. An increased electrical conductivity after spiking refl the displacement of indigenous, exchangeable alkaline earth cations (Ca, Mg, K, and Na) in the soil by the added metal. Elevated salinity can increase metal bioavailability through the effects of cation competition and changes in soil surface potential [1].

To account for possible effects of salinity, one option is to compensate for differences in counterion concentrations between treatments with another salt having a cation that is not toxic at the tested concentrations [2]. Another possibility is to perform additional tests to assess the toxicity of the counterion [3]. Both approaches might be appropriate when one needs to account for additional toxicity of the counterion; however, if interactions (synergistic or antagonistic) occur between metal and counterion toxicity, then both approaches are inappropriate. Furthermore, when salinity affects metal partitioning, as found by Stevens et al. [4], these approaches have limitations. Leaching the spiked soils before conducting toxicity assays is probably a better method to account for the effect of the counterion [5]. Alternatively, an organic counterion can be used that mineralizes after spiking [6]. In the latter method, however, the equilibrium period has to be long enough to allow mineralization of the counterion. Such mineralization probably can be seriously delayed at high metal concentrations, and changes in soil pH might occur.

Lead toxicity in contaminated soils decreases after leaching [4,7]. However, it is unclear if metal bioavailability in leached spiked soils is comparable to the bioavailability observed in historically contaminated fi soils. The difference between metal bioavailability in freshly spiked soils and historically contaminated fi soils, often called aging, has been reviewed by Lock and Janssen [8]. Being able to predict metal toxicity in historically contaminated soils is more challenging but would be very useful in the risk assessment of metal-contaminated soils and the derivation of soil-quality criteria. In the present study, Pb toxicity therefore was assessed simultaneously in soils historically contaminated with Pb and in leached as well as unleached soils spiked at similar Pb concentrations in the laboratory.

## MATERIALS AND METHODS

### *Test organisms*

The culture of *Folsomia candida* Willem, 1902 was obtained from Aquasense (Amsterdam, The Netherlands). Animals were cultured on a substrate of plaster of Paris and pulverized chemical-activated charcoal in a ratio of 8:1 (w/w). Granulated dry yeast was added weekly as a food source. The culture has been maintained in our laboratory for at least 10 years at 20°C. Because *F. candida* is blind and lives in the soil, cultures were maintained in complete darkness. Culturing these animals at constant lighting of 400 to 800 lux, as suggested by the International Organization for Standardization (ISO) [9], is a waste of energy.

* To whom correspondence may be addressed (koen_lock@hotmail.com).

*Toxicity assays*

Tests with the springtail *F. candida* were carried out according to ISO 11267 [9]. Ten 10- to 12-d-old synchronized springtails were exposed per glass vessel containing 30 g wet weight of soil. Four replicates were used per exposure concentration. Reproduction tests with *F. candida* took four weeks to complete. During exposure, vessels were kept at 20°C and a 16:8-h light:dark cycle at 400 to 800 lux. Soil moisture content was adjusted twice a week by replenishing weight loss with the appropriate amount of deionized water. Granulated dry yeast was added weekly on the soil surface as a food source. At the end of the test, juveniles were counted after fl The number of juveniles in the controls always exceeded the prescribed minimum of 100 instars per vessel. Mortality in the controls never exceeded 10%.

*Test substrates*

Soils were sampled along three industrially contaminated transects characterized by a gradient in the total Pb concentration in the soil. Care was taken to choose soils that contained very high Pb concentrations but with as little contamination by other substances as possible. Measurements of Zn, Cd, Cu, and Ni concentrations revealed that the sites were, indeed, mainly contaminated with Pb (data not shown). The Zn concentrations increased up to 750 mg/kg dry weight only in the transect points at site A. However, no adverse effects were observed at the transect soils of site A (see below), indicating that the high Zn concentrations did not affect the reproduction of *F. candida*. For each transect, six Pb-contaminated and one control soil were sampled according to a composite sampling procedure. After sampling, soils were air-dried and sieved (mesh size, 2 mm) to avoid changes in bioavailability as a result of reduction and to freeze bacterial activity. The pH of the soils sampled in transect A ranged from 6.2 to 6.9 and was adjusted to 7.3, and the fourth Pb concentration of transect B (5,550 mg/kg dry wt) with a pH of 6.3 was adjusted to 7.2 using $CaCO_3$ (purity, >99%; VWR, Leuven, Belgium).

One day after spiking with $Pb(NO_3)_2$ (purity, >99%; Chem-Lab, Zedelgem, Belgium), leaching was performed on the control soils that were spiked at the same total Pb concentrations as measured in the respective transect soils. The soils were leached with two pore volumes of artifi rainwater and drained overnight with artifi rainwater composed of $CaCl_2$ (5 X $10^{-4}$ M), $Ca(NO_3)_2$ (5 X $10^{-4}$ M), $MgCl_2$ (5 X $10^{-4}$ M), $Na_2SO_4$ ($10^{-4}$ M), and KCl ($10^{-4}$ M) with a pH of 5.9 [10]. The leached soils were air-dried and then sieved again (mesh size, 2 mm).

Toxicity assays were conducted according to a full-factorial design with three soils (A, B, and C) and three treatments (spiking, spiking and leaching, and transect). One week before the toxicity assays, samples of the control soils were spiked with $Pb(NO_3)_2$ (purity, >99%; Chem-Lab) at the same total Pb concentrations as measured in the respective transect soils. Transect soils and leached soils were moistened one week before toxicity testing. The soils of all treatments were adjusted to a soil moisture content of 31% for soil A, 30% for soil B, and 23% for soil C to obtain a crumbly structure.

*Metal analysis*

Pore water was collected after a one-week equilibration period following the spiking procedure, which was performed concurrently with the start of the toxicity assays. Pore water was sampled by the double-chamber centrifugation method (20 min at 3,000 *g*) and fi through a 0.45-μm membrane fi (Millipore, Brussels, Belgium) [10]. Immediately after centrifugation, pore-water pH was determined, and Pb concentrations in the pore water were measured with inductively coupled plasma–optical emission spectroscopy (ICP-OES; Optima 3300 DV; Perkin-Elmer, Norwalk, CT, USA). Pore-water Pb concentrations below the detection limit (0.008 mg/L) were set at the detection limit divided by two (0.004 mg/L). Total metal concentrations in the soil were determined by aqua regia destruction [11] followed by ICP-OES. A reference soil (Community Bureau of Reference, Brussels, Belgium) was used as certifi reference material.

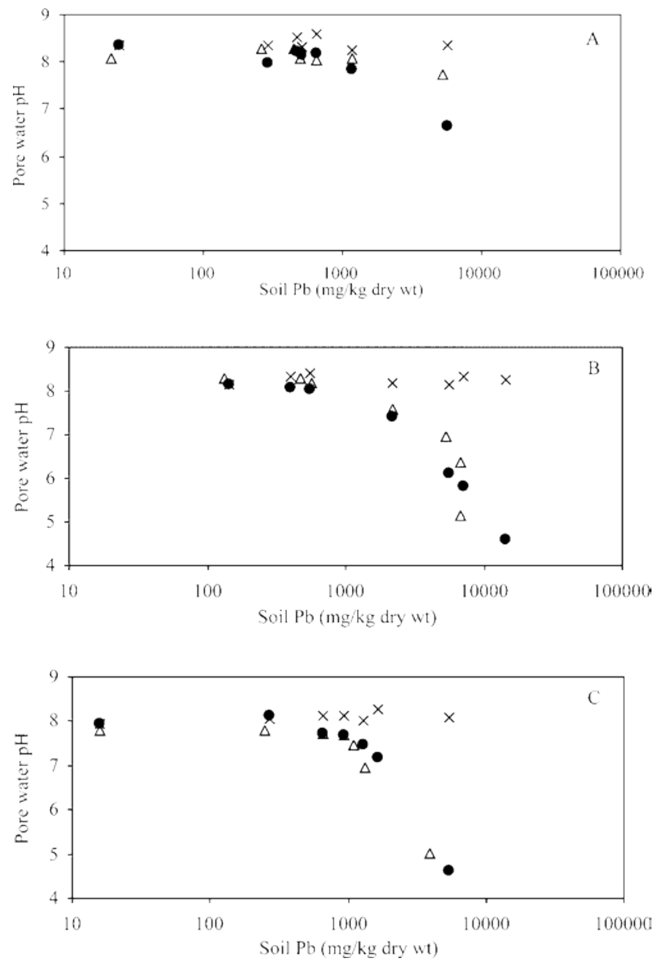


Fig. 1. Pore-water pH versus total Pb concentration in soils A, B, and C. e = spiked; Lo = spiked and leached; X = transect.

*Statistics*

The effect concentrations causing 50% inhibition (EC50s) were calculated using the moving average method [12]. No-observed-effect concentrations and lowest-observed-effect concentrations were calculated by Kruskal–Wallis analysis of variance followed by post-hoc multiple comparisons [13].

**RESULTS**

Pore-water pH dropped by as much as three pH units or more when the three soils were spiked at high Pb concentrations (Fig. 1). Leaching decreased this change in pH only slightly (Fig. 1). Pore-water Pb concentrations increased with increasing spiked total Pb concentrations in the soil, but leach-

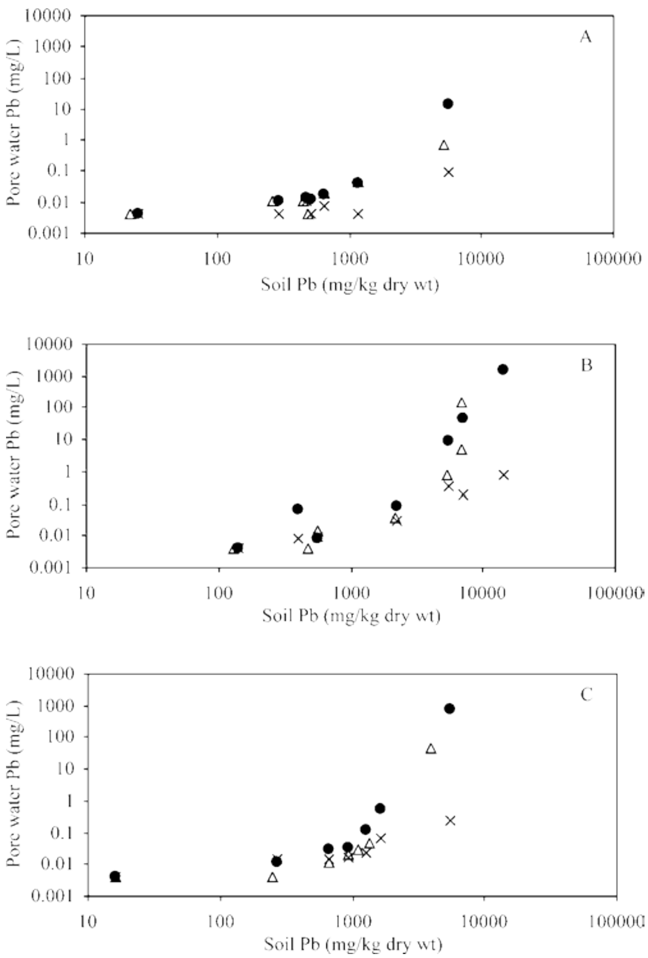


Fig. 2. Pore-water Pb concentration versus total Pb concentration in soils A, B, and C. e = spiked; Lo = spiked and leached; X= transect.

ing reduced the pore-water Pb concentrations at the highest added Pb concentrations (Fig. 2). Despite similar total Pb concentrations in the soil, both leached and unleached soils that were spiked with Pb contained much higher pore-water Pb concentrations compared with those of the historically contaminated transect soils (Fig. 2).

Leached and unleached Pb-spiked soils all had similar 28-d EC50s for *F. candida*, ranging from 2,060 to 3,210 mg/kg dry weight (Table 1). However, in the historically contaminated transect soils, the reproduction of *F. candida* was never reduced by more than 50%, even though these soils contained total Pb concentrations similar to those of the spiked soils. When expressed as pore-water Pb concentrations, 28-d EC50s for *F. candida* ranged from 0.0663 to 3.00 mg/L in the spiked soils (Table 1).

In the spiked soils, total Pb concentrations of 2,207 mg/kg dry weight or lower never had a signifi effect on the reproduction of *F. candida*, whereas Pb concentrations of 3,877 mg/kg dry weight or higher always caused a signifi decrease in reproduction (Table 2). In the historically contaminated transect soils, however, reproduction was signifi affected only in one soil of transect B, containing 14,436 mg Pb/kg dry weight (Table 2). When expressed as pore-water Pb concentrations, reproduction was never affected at concentrations of 0.539 mg/L or lower, whereas reproduction was always signifi reduced at concentrations of 0.678 mg/L and higher, irrespective of the soil treatment (Table 2).

## DISCUSSION

Despite the use of different soils, EC50s reported in the literature for *F. candida* exposed to Pb-spiked soils are similar to those reported in the present study. No signifi effects on the reproduction of the springtail *Proisotoma minuta* were observed when exposed to Arthursleigh soil (pH 4.88; cation-exchange capacity, 3.65 $cmol_c$/kg) spiked at Pb concentrations of up to 3,000 mg/kg dry weight [14]. Sandifer and Hopkin [15,16] reported 28-d EC50s for *F. candida* of 2,970, 1,360, and 3,160 mg/kg dry weight of Pb in Organization for Economic Co-operation and Development (OECD) soils exposed at 20°C and set at pH 6, 5, and 4.5, respectively, and a 28-d EC50 of 1,570 mg/kg dry weight of Pb when exposed at 15°C and set at pH 6. Bongers et al. [7] exposed *F. candida* to Pb in Landwirtschaftliche Untersuchungs und Forschungsanstalt Speyer (LUFA) 2.2 soil (pH 6.1; cation-exchange capacity, 9 $cmol_c$/kg) and found a 28-d EC50 (with 95% confi intervals) of 1,900 (1,100–2,800) mg/kg dry weight when spiked with $PbCl_2$ and a 35-d EC50 of 580 (460–700) mg/kg dry weight when spiked with $Pb(NO_3)_2$. After leaching, however, a 28-d EC50 of 2,400 (1,500–3,200) mg/kg dry weight when spiked with $PbCl_2$ and a 35-d EC50 of 1,700 (1,100–2,300) mg/kg dry weight when spiked with $Pb(NO_3)_2$ were found [7]. The latter approach does not allow assessment of the effect of the counterion used; however, it could be concluded that leaching decreased Pb toxicity.

Table 1. Twenty-eight day effect concentrations causing 50% inhibition (EC50s) for *Folsomia candida* exposed to Pb in a factorial design with three soils (A, B, and C) and three treatments (spiked, spiked and leached, and transect)[a]

| Soil | Treatment | 28-d EC50 (mg/kg dry wt) | 28-d EC50 (mg/L) |
|---|---|---|---|
| A | Spiked | 2,570 (2,380–2,780) | 0.744 (0.255–2.17) |
| | Spiked and leached | 2,060 (1,890–2,270) | 0.144 (0.0756–0.250) |
| | Transect | >5,690 | >0.090 |
| B | Spiked | 3,210 (3,030–3,380) | 0.596 (0.186–1.63) |
| | Spiked and leached | 2,580 (1,540–3,330) | 0.0663 (0.0114–0.157) |
| | Transect | >14,400 | >0.739 |
| C | Spiked | 2,160 (1,220–2,980) | 3.00 (0.0936–20.9) |
| | Spiked and leached | 2,320 (2,120–2,540) | 1.68 (0.157–20.4) |
| | Transect | >5,460 | >0.235 |

[a] The EC50s (with 95% confi intervals in parentheses) are expressed as total soil concentrations (mg/kg dry wt) and as pore-water concentrations (mg/L).

Table 2. The no-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs) for *Folsomia candida* exposed to Pb in a factorial design with three soils (A, B, and C) and three treatments (spiked, spiked and leached, and transect)[a]

| Soil | Treatment | 28-d NOEC (mg/kg dry wt) | 28-d LOEC (mg/kg dry wt) | 28-d NOEC (mg/L) | 28-d LOEC (mg/L) |
|---|---|---|---|---|---|
| A | Spiked | 1,163 | 5,686 | 0.041 | 13.5 |
| | Spiked and leached | 1,173 | 5,211 | 0.048 | 0.678 |
| | Transect | 5,686[b] | | 0.090[b] | |
| B | Spiked | 2,207 | 5,550 | 0.083 | 9.5 |
| | Spiked and leached | 2,193 | 5,345 | 0.038 | 0.785 |
| | Transect | 7,091 | 14,436 | 0.363 | 0.739 |
| C | Spiked | 1,630 | 5,457 | 0.539 | 811 |
| | Spiked and leached | 1,331 | 3,877 | 0.045 | 49 |
| | Transect | 5,457[b] | | 0.235[b] | |

[a] The NOECs and LOECs are expressed as total soil concentrations (mg/kg dry wt) and as pore-water concentrations (mg/L).
[b] Unbound NOEC.

The drop in pore-water pH after spiking with high Pb concentrations and only a slight rise in pH after percolation also were observed by Bongers et al. [7] and by Stevens et al. [4]. These shifts in pH might not cause direct effects on the performance of the test organisms, but changes in pH can affect metal bioavailability. Stevens et al. [4] also reported an increase in electric conductivity when soils were spiked with Pb, and the electric conductivity dropped again after leaching. Thus, it can be concluded that addition of soluble metal salts causes shifts in both pH and soil solution ionic strength, both of which can affect metal partitioning.

According to Bongers et al. [7], the decrease in Pb toxicity after leaching was caused by the reduction in toxicity of the counterions, because those authors did not fi differences in the water-soluble and the 0.01 M $CaCl_2$–extractable Pb concentrations between leached and unleached soils. However, these Pb fractions do not necessarily correspond to the fraction that is bioavailable to *F. candida*, and the difference in toxicity might, for example, result from differences in salinity or pH, both of which can affect Pb bioavailability. Stevens et al. [4] exposed lettuce (*Lactuca sativa*) in fi different fi soils spiked with Pb. In three of the fi soils, leaching increased the Pb EC50 signifi , whereas in the two other soils, the EC50 did not change. It was indicated that the shift in EC50 was not caused by the toxicity of the used nitrate ion but, rather, was an indirect effect of the salinity, increasing Pb concentrations in soil solution and increasing Pb bioavailability for a given total metal concentration. Similar critical Pb concentrations in the plant tissue across all treatments supported this hypothesis [4]. Stevens et al. [4] needed two to fi pore volumes to obtain a conductivity of less than 1.2 dS/m. Van Gestel and Koolhaas [17] also indicated that fl their soils with two pore volumes was insuffi because conductivities of up to 4,150 μS/cm were observed. Therefore, the two pore volumes used for the leaching procedure in the present study might have been insuffi

Although Bongers et al. [7] stated that the water-soluble and 0.01 M $CaCl_2$–exchangeable Pb fractions were not altered by leaching, it can be observed from their graphs that, especially at the highest Pb concentrations, the water-soluble fraction decreased after leaching. Stevens et al. [4] found no differences in 1 M ammonium nitrate–extractable Pb between leached and unleached soils. However, in the same fi fi soils, 1 M ammonium nitrate–extractable Pb fractions and isotopic dilution techniques showed no signifi differences in terms of labile metal pools between leached and unleached soils, whereas water-soluble Pb concentrations decreased after leaching [4]. Also, in OECD soil, no differences were observed in 0.01 M $CaCl_2$–extractable Zn concentrations, whereas water-soluble Zn concentrations dropped after leaching combined with an extended equilibrium period of two months [18]. These results indicate that water-soluble metal concentrations can decrease after leaching, whereas no differences are detected with stronger extraction techniques, such as extraction with 1.1 M $CaCl_2$, 1 M ammonium nitrate, and aqua regia. Also, in the present study, a decrease in the pore-water Pb concentrations was observed after leaching.

Despite the fact that very high Pb concentrations had to be spiked to reduce the reproduction of *F. candida* by 50%, similar total Pb concentrations in historically contaminated fi soils did not affect reproduction of *F. candida* to the same extent, and even higher concentrations were needed to reduce the reproduction by 50%. The results obtained indicate that leaching of metal-spiked soils can account only in part for differences in metal bioavailability. Because aging is a complicated process [8], this is not surprising. However, when metal toxicity data were expressed as pore-water concentrations, differences between leached as well as unleached spiked soils and historically contaminated fi soils could be explained, at least in part. One drawback might be that EC50s expressed as pore-water Pb concentrations had bigger confi dence intervals, which resulted from the greater relative differences between successive pore-water Pb concentrations as opposed to total Pb concentrations.

It can be concluded that leaching metal-spiked soils may reduce, but cannot fully account for, the difference in metal bioavailability between spiked soils and historically contaminated fi soils. However, in agreement with Smit and Van Gestel [5], the present study found that when metal toxicity is expressed as pore-water concentrations instead of total soil concentrations, differences in metal bioavailability could be explained.

*Acknowledgement*—This research was supported by the International Lead Zinc Research Organization and by Lead Development International.

## REFERENCES


1. Garcia-Miragaya J, Page AL. 1976. Infl of ionic strength and inorganic complex formation on the sorption of trace amounts of Cd by montmorillonite. *Soil Sci Soc Am J* 40:658–663.
2. Posthuma L, Baerselman R, Van Veen RPM, Dirven-Van Breemen EM. 1997. Single and joint effect of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. *Ecotoxicol Environ Saf* 38:108–121.
3. Schrader G, Metge K, Bahadir M. 1998. Importance of salt ions in ecotoxicological tests with soil arthropods. *Appl Soil Ecol* 7: 189–193.
4. Stevens DP, McLaughlin MJ, Heinrich T. 2003. Determining toxicity of lead and zinc runoff in soils: Salinity effects on metal partitioning and on phytotoxicity. *Environ Toxicol Chem* 22: 2017–3024.
5. Smit CE, Van Gestel CAM. 1998. Effects of soil type, prepercolation, and aging on bioaccumulation and toxicity of zinc for the springtail *Folsomia candida*. *Environ Toxicol Chem* 17:1132–1141.
6. Chauvat M, Ponge J. 2002. Colonization of heavy metal–polluted soils by Collembola: Preliminary experiments in compartmented boxes. *Appl Soil Ecol* 21:91–106.
7. Bongers M, Rusch B, Van Gestel CAM. 2004. The effect of counterion and percolation on the toxicity of lead for the springtail *Folsomia candida* in soil. *Environ Toxicol Chem* 23:195–199.
8. Lock K, Janssen CR. 2003. Infl of aging on metal availability in soils. *Rev Environ Contam Toxicol* 178:1–21.
9. International Organization for Standardization. 1999. Soil quality—Effects of soil pollutants on Collembola (*Folsomia candida*): Method for determination of effects on reproduction. ISO 11267. Geneva, Switzerland.
10. Oorts K, Bronckaers H, Smolders E. 2006. Discrepancy of the microbial response to elevated copper between freshly spiked and long-term contaminated soils. *Environ Toxicol Chem* 25:845–853.
11. Houba VJG, Van der Lee JJ, Novozamsky I, Walinga I. 1989. Soil and plant analysis, a series of syllabi. Part 5: Soil Analysis Procedures. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands.
12. Stephan CE. 1977. Methods for calculating an LC50. In Mayer FL, Hamelink JL, eds, *Aquatic Toxicology and Hazard Evaluation*. STP 634. American Society for Testing and Materials, Philadelphia, PA, pp 65–84.
13. Conover WJ. 1980. *Practical Nonparametric Statistics*. John Wiley, New York, NY, USA, pp 229–237.
14. Nursita AI, Singh B, Lees E. 2005. The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola). *Ecotoxicol Environ Saf* 60:306–314.
15. Sandifer RD, Hopkin SP. 1996. Effects of pH on the toxicity of cadmium, copper, lead, and zinc to *Folsomia candida* Willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere* 33:2475–2486.
16. Sandifer RD, Hopkin SP. 1997. Effects of temperature on the relative toxicities of Cd, Cu, Pb, and Zn to *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf* 37:125–130.
17. Van Gestel CAM, Koolhaas JE. 2004. Water-extractability, free ion activity, and pH explain cadmium-sorption and toxicity to *Folsomia candida* (Collembola) in seven soil-pH combinations. *Environ Toxicol Chem* 23:1822–1833.
18. Lock K, Janssen CR. 2002. The effect of aging on the toxicity of zinc for the potworm *Enchytraeus albidus*. *Environ Pollut* 116:289–292.