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Assessment of the bioavailability and toxicity of lead polluted soils using a combination of chemical approaches and bioassays with the collembolan *Folsomia candida*

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HIGHLIGHTS

- Soils from different landscapes of a shooting range are examined.
- Bioavailability of Pb varies with soil properties especially, pH and OM.
- Collembolan reproduction was more sensitive to pH than to Pb in soils.
- Collembolans avoided acid Pb contaminated soils.

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ABSTRACT

Understanding bioavailability and toxicity is essential for effective ecological assessment of contaminated soils. Total, water and 0.01 M $CaCl_2$ extractable and porewater Pb concentrations and soil properties in different shooting field soils were investigated. Three artificial soils containing different pH and organic matter contents and two natural soils were included as controls. Survival, reproduction and avoidance responses of *Folsomia candida* exposed to these soils as well as internal Pb concentrations were measured. In the shooting range soils, total Pb concentrations were 47–2398 mg/kg dw, pH_{CaCl_2} 3.2–6.8 and organic matter content 3.8–7.0%. Pb concentrations in *F. candida* linearly increased with increasing Pb concentrations in the soils. Acid forest soils caused significantly higher collembolan mortality and avoidance responses and significantly lower reproduction than the neutral grassland soils, which could be attributed to differences in pH and especially $CaCl_2$ extractable Pb concentrations. Soil properties significantly affected bioavailability and toxicity of Pb, but overall the collembolans seemed more sensitive to pH than to Pb in soils. This study shows the importance of selecting proper reference soils for assessing the effects of field soils.

1. Introduction

Lead-contaminated land is a global environmental problem that results not only in ecological and human health risks, but can also have large financial implications in terms of remediation costs and re-development issues [1]. Shooting ranges, used for both military training and sport, can constitute an important source of lead (Pb) contamination in terrestrial ecosystems due to the use of ammunition pellets containing Pb [2]. Because of its widespread use and its possible irreversible impairment of ecological soil processes, environmental risks associated with Pb contamination at shooting ranges are receiving increased interest from various national and international regulatory organizations [3].

Pb availability measured using extractants such as weak salts or weak acids may provide a first indication of the potential risk [4]. However, the uncertainty associated with the performance of extractants under various environmental conditions hinders endorsement of single or sequential extraction procedures. To address the relevance of chemical extraction methods for risk assessment, the analytically extracted metal concentrations need to be interpreted in a biological context [5]. There is an increasing need to incorporate bioassays in risk assessments and hazard identification [6,7]. Bioassays with soil animals however, may also present some problems: acute tests, for instance, do not provide an

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insight into the effects of the contaminant on population dynamics while chronic tests are time-consuming and labor intensive [8]. In acute toxicity tests, some soil invertebrate species proved to be very resilient, with an "avoidance mechanism" that distorted the results toward lower toxicity values than expected [9]. As an alternative to the classic survival or reproduction tests therefore sub-lethal avoidance behavior tests have been developed as ecologically relevant, sensitive, rapid, and cost-effective first screening tools for assessing soil contamination [9–11].

Since earthworms avoid Pb treated soil (1000–10,000 mg Pb/kg dw) in a dose-related manner and prefer neutral to slightly acidic soils (pH tolerance: 4.0–9.0), they may not be suitable for testing more acidic soils [12,13]. Soil dwelling collembolans are considered ecologically relevant species for ecotoxicological tests because they represent arthropods that have a different route of exposure and have a wider pH tolerance (3.2–7.7) compared to the earthworm species commonly used in toxicity tests [12,13]. The collembolan *F. candida* is often used as bioindicator for pollution due to its widespread distribution in different soil types, flexibility of its alimentary habits, its locomotor ability, and its role in the decomposition of organic matter, regulation of microbial activity and nutrient cycling [10,14].

Terrestrial ecotoxicity not only varies between species but soil characteristics also greatly influence the effect of metals by altering bioavailability [15]. In previous studies, the effect of aging on the bioavailability of Pb was hardly taken into account. Because most toxicity tests were carried out in freshly spiked soils, the effect concentrations may be regarded as conservative [13]. When combined with environmental chemistry of naturally aged soils, bioassays (including avoidance tests) provide more complete and relevant information on contaminant bioavailability in soils and, therefore a more meaningful and holistic risk assessment of contaminated sites [9,16]. To considerably improve site-specific risk assessment of Pb-contaminated land, it is highly recommended to combine soil characterization, chemical extraction methods and bioassays [7,9,10].

This study is part of a broader investigation that aimed at assessing the ecotoxicological risk of Pb in shooting range soils and deals with the effects on springtails; earlier studies described the effects on earthworms and enchytraeids [17,18]. Six natural field soils were collected from different landscapes (bullet plot, forest and grass lands) of a shooting field in the Netherlands, representing a gradient of Pb pollution but also different pH and organic matter contents. To elucidate effects of main soil properties on collembolans, three artificial reference soils with different pH and organic matter contents were used for comparison. Relationships between the survival and reproduction of *F. candida* and the total and water- and $CaCl_2$-extractable and porewater Pb concentrations in soil as well as internal Pb concentrations in the surviving collembolans were determined. In addition, the behavioral response of *F. candida* to Pb in the shooting field soils was also investigated. The final aim of this study was to combine conventional chemical analysis, long-term toxicity tests and short-term avoidance tests to support the environmental risk assessment of Pb in shooting field soils.

2. Materials and methods

2.1. Soil sampling

Six natural soils were sampled from forest (F), grassland (G) and bullet plot (B) fields, which represent three landscapes of a shooting field in the Netherlands. Assuming it had similar soil properties without being contaminated, a soccer field soil (S) near the shooting range was sampled as a reference for the survival and reproduction tests. Three artificial soils (R1, R2, R3) were prepared based on OECD artificial soil [19] in order to "mimick" the shooting field soils in pH and organic matter content, assuming these properties had more effect on Pb toxicity than soil texture. A standard artificial soil (R1) were prepared by mixing 10% finely ground sphagnum peat (<1 mm), 20% kaolin clay, and 70% quartz sand (dry weight) and adjusting nominal pH_{CaCl_2} to 6.0 ± 0.5 by adding $CaCO_3$. The other two artificial soils were prepared by reducing peat contents to 5% (R2) or 2.5% (R3), increasing sand content accordingly and adjusting nominal pH_{CaCl_2} to 3.5 (R2) or 6.5 (R3) with $CaCO_3$. The standard natural LUFA 2.2 soil (LF2.2; LUFA-Speyer, Sp 2121, Germany) was used as an additional control of the performance of the test animals.

For a full description of the methods used to analyze the soils, it can be referred to Luo et al. [17].

2.2. Survival and reproduction toxicity tests

Before starting the tests, all soils were dried completely at 50 °C and subsequently moistened to 50% of their water holding capacity (WHC). *F. candida* ("Berlin strain"; VU Amsterdam) were cultured in plastic containers with a moist bottom of plaster of Paris containing 10% charcoal, at 20 °C at a light/dark regime of 12/12 h. The experiment was initiated with 10–12 day old juveniles that were obtained by synchronizing the egg laying of the culture animals, fed with dried baker's yeast (Dr. Oetker). Tests with *F. candida* were carried out according to ISO guideline 11267 [20], using 100 ml glass test containers containing 30 g moist soil each. Five replicates were prepared for each test, reference and control soil. At the start of the test, ten synchronized animals were transferred into each test jar and fed a few grains of dried baker's yeast. The jars were incubated in a climate room at 20 ± 1 °C and at a light/dark regime of 12/12 h. Once a week, the moisture content of the test soils was maintained by adding Milli-Q. After 28 days exposure, the content of each jar was flushed into a 300 ml beaker glass using 100 ml water. The mixture was gently stirred to let all surviving adults and juveniles produced floating on the water surface. A picture of the water surface was taken and the number of offspring was counted using digital imaging software.

Surviving adults were carefully taken from the water surface and allowed to remove excess water and empty their guts by placing them for a few minutes on a dry bottom of plaster Paris. After freeze-drying and determination of their dry weight, the animals were individually digested in a 300 μl $HClO_4/HNO_3$ mixture (1:7 v/v; Ultrex grade, Baker) and Pb concentrations in their bodies were measured using a Perkin Elmer 5100 Atomic Absorption Spectrometer equipped with a graphite furnace assembly. Quality of the analysis was controlled by analyzing certified reference material (Dolt 4). Pb recoveries were 85–115% of the certified value.

Bioaccumulation factors (BAFs) were calculated as the ratio of Pb concentrations in the collembolans and total Pb concentrations in the soil.

2.3. Avoidance test

The procedure adopted in the avoidance tests was based on International Organization for Standardization (ISO) [21] with some modifications. Cylindrical plastic boxes (diameter, 5 cm; depth, 2 cm) were divided into two equal sections by means of a card divider introduced vertically. Ten grams (dry weight equivalent) of a shooting field soil was placed in one of the sections, while the other one was filled with control soil (LF2.2) or a reference soil (R1/R2/R3). Five replicates were tested for each field soil–control/reference combination. After removing the plastic divider, 10 individuals (10–12 days old and without visible signs of damage) were added to each test vessel. To limit water