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Orthonychiurus pseudostachianus (collembola) as a toxicity test organism and selection of an ecotoxicological test battery to assess soil quality

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ABSTRACT

This study aimed at developing a test using an autochthonous collembolan species (*Orthonychiurus pseudostachianus*, Gisin 1956) to assess soil toxicity. To evaluate the feasibility of this species in soil ecotoxicity assessment, it was considered whether the biological characteristics of the proposed species met the criteria reported in OECD guideline 232 for toxicity testing using *Folsomia candida*. Next, the sensitivity to soil metal contamination was evaluated performing the test on an artificial soil spiked with Zn concentrations ranging from 4.79 to 479 $\mu g\,g^{-1}$ d.w. To verify its suitability for soil ecotoxicity assessment, the proposed test was performed on soils collected at six sites in the urban area of Naples together with other ecotoxicological tests. The other aim of the study was to identify the lowest number of tests needed to obtain an optimized test battery for soil ecotoxicity assessment. At the tested Zn concentrations, no mortality of *O. pseudostachianus* was observed whereas reproduction was halved at 37.4 $\mu g\,Zn\,g^{-1}$ d.w. (95% confidence limits 29.3–44.8). In the field-collected soils, reproduction of *O. pseudostachianus* proved a useful tool to assess soil quality. A test battery composed only by *Sinapis alba* (germination index), *O. pseudostachianus* (reproduction), *Eisenia veneta* (body growth), and *Heterocypris incongruens* (body growth) tests were shown to be sufficiently informative to assess soil toxicity.

1. Introduction

In recent years, there has been growing concern about the toxic effects on organisms caused by chemical substances entering the soil system (Scott-Fordsmand et al., 1999; Xu et al., 2009). Metals are amongst the most abundant soil pollutants and are not biodegradable, so they can accumulate strongly affecting biodiversity, functionality and activity of terrestrial ecosystems (Wong et al., 2006). Additionally, the excessive accumulation of heavy metals in soils may pose serious risks to human health (Chen et al., 1992; Nordberg, 1996; Cui et al., 2005).

To perform only a single species test could not provide a full picture of soil toxicity. In fact, it could reflect only the sensitivity of the species and then over- or under-estimate the overall soil toxicity (Davoren and Fogarty, 2004). To better highlight realistic soil toxicity, a battery of toxicity tests using various species and investigating different endpoints is needed (Davoren and Fogarty, 2004). Plants, earthworms, ostracods and collembolans are very often used in ecological studies and in ecotoxicological risk assessment for terrestrial ecosystems (Hamdi et al., 2006; Hubálek et al., 2007). Plants have a relevant ecological role being primary producers; the ostracods are representative herbivorous invertebrate species, and earthworms and collembolans play a key role in organic matter decomposition, nutrient cycling and energy flow.

Unfortunately, few soil species are used in standard ecotoxicity tests compared to the huge variety of soil dwelling species. Therefore, it is desirable to increase the battery of contact tests in soil ecotoxicity assessment with autochthonous, sensitive and representative species.

Collembolans show species-specific differences in heavy metal sensitivity: some species decrease in abundance along a pollution gradient, whereas others maintain or even increase their population (Filser et al., 2000; Heupel, 2002). Amongst collembolans used in standard ecotoxicity tests, *Folsomia candida* is widespread. Nevertheless, several authors suggest to use indigenous collembolan species because they greatly increase the ecological relevance and reliability of laboratory tests (Greenslade and Vaughan, 2003; Son et al., 2007; Nakamori et al., 2008).

The aim of this research was to propose a nonstandard collembolan species, *Orthonychiurus pseudostachianus*, as toxicity test organism and to check its contribution to the assessment of soil toxicity. Unlike *F. candida* that is a cosmopolitan collembolan, *O. pseudostachianus* is autochthonous of Europe and widespread mostly in Italy, and it can give a more detailed response to our environment.

Therefore, to evaluate the feasibility of this species in soil ecotoxicity assessment, we considered: (1) whether the biological characteristics of the proposed species met the criteria suggested

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Fig. 1. Individual of *Orthonychiurus pseudostachianus* (Gisin), 40× magnification.

in the OECD guideline 232 for *F. candida* (OECD, 2009), and (2) its sensitivity to metals, performing the test on Zn-spiked soils (Zn was selected as indicator contaminant). In addition, the proposed test was performed on soils collected at six sites in the urban area of Naples together with ecotoxicological tests with plants, ostracods and earthworms in order to check its sensitivity to different natural soils. Finally, multivariate statistical analyses were performed in order to select the lowest test number, amongst the performed ones, that might guarantee the same result with lower costs and efforts.

2. Materials and methods

2.1. O. pseudostachianus culture and toxicity test

In order to increase the battery of ecotoxicity standard tests, organisms belonging to the species *O. pseudostachianus* (*Onychiuridae* family) were selected (Fig. 1). These collembolans are soil and litter dwelling, are completely depigmented and have no jumping organs (Greenslade and Rusek, 1996). To recognize the native collembolan species, the taxonomic key reported by Pomorski (1998) was used.

In order to define the biology and life history of *O. pseudostachianus*, 50 juveniles (10–12 days old) were isolated in boxes set up as described below and monitored daily until death. They reproduce parthenogenetically; the eggs are spherical, whitish, opaque and shiny; juveniles morphologically differ from adults (2.0–2.5 mm long) in proportion and size.

Starting from the consideration that *O. pseudostachianus* is a terrestrial collembolan like *F. candida*, its culture conditions were set according to the standard protocol for *F. candida* (OECD, 2009). Standard artificial soil (OECD, 1984) was moistened by adding deionised water to reach approximately 50% of its water holding capacity (WHC). One aliquot of this soil was used for the *O. pseudostachianus* culture, whereas another aliquot was divided into batches to be used for the tests. Briefly, *O. pseudostachianus* were grown in plastic boxes containing the moist OECD artificial soil, at 20 ± 1 °C and at a light:dark photoperiod of 16 h:8 h (400 lux). *O. pseudostachianus* were fed with dry yeast once a week.

In order to obtain specimens at the same development stage and to promote the oviposition, adults were transferred in plastic pots containing a mix of plaster of Paris, charcoal and water (8:1:9 = w:w:w) at constant moisture. After oviposition, the adults were removed and ten juveniles (12–14 days old) were used for the toxicity tests (OECD, 2009). To perform the test, the selected juveniles were added to the OECD artificial soil that moistened to 50% of the WHC (corresponding with a moisture content of 29%) and kept at the same culture conditions for 28 days.

After verifying that *O. pseudostachianus* were growing well at the culture conditions applied, its response to soil metal contamination was evaluated by exposure to OECD artificial soil spiked with $ZnCl_2$ (CAS no. 20,808-6, purity >98%, Sigma–Aldrich) solution. Zn was chosen because several authors (Lock and Janssen, 2003; Xu et al., 2009) reported that collembolans are already affected at Zn soil concentrations of 390–400 $\mu g\,g^{-1}$ d.w. In particular, zinc affected *F. candida* reproduction (Smit et al., 2004) and increased its juvenile period upon exposure in food (Fountain and Hopkin, 2001). The day before starting the test, the Zn solutions were manually mixed to each batch of premoistened OECD soil, for 30 min. Nominal $ZnCl_2$ concentrations were: 0, 10, 100, 200, 400, 1000 $\mu g\,Zn\,g^{-1}$ d.w. soil. Zn concentrations, measured by flame atomic absorption spectrometry after acidic digestion (see Section 2.2), were 0.00 – 4.79 – 47.9 – 95.8 – 192 – 479 $\mu g\,g^{-1}$ d.w.

For the test, once a week each pot was opened for aeration, for replenishing the lost water, and for feeding the collembolans with 2 mg of dry yeast. At the end of the 28-day exposure period a large amount of deionised water was added at each pot and the soil was stirred to make all individuals floating to the surface. Next, the specimens were observed using a stereomicroscope (Zeiss, Stemi 2000-C), 40× magnification, to count both juveniles and adults. Survival was recorded as recovered adults whereas reproduction was expressed as the number of juveniles produced. The test was performed following the standard protocol for *F. candida* (OECD, 2009), using five replicate pots per concentration. The EC_{50} (i.e. Zn concentration that caused 50% effect compared with the non-treated control) was calculated by the Inhibition concentration approach, Icp (Norberg-King, 1993), using a linear interpolation method.

2.2. Soil sampling, and soil chemical–physical characterization

At six sites in the urban area of Naples, at least ten samples of surface soils (0–10 cm), after litter removal, were collected. The soils were collected under *Quercus ilex* L. trees, widespread in the studied area, in order to minimize the variation in the effect of vegetation upon soil characteristics.

In the laboratory, the soils were sieved (2 mm) and characterized for pH, water holding capacity (WHC), organic matter content (OM) and texture. pH was measured in a soil:distilled water suspension (1:2.5 = v:v) by electrometric method (Allen, 1989); soil WHC was estimated as the soil water content after saturation followed by leaching of gravitational water (Allen, 1989), and organic matter content was evaluated by loss of weight after ignition at 550 °C for 2 h (Allen, 1989). Soil textural analysis was performed by both wet sieving (particles > 50 μm) and X-ray sedigraphy (particles < 50 μm) using a sedigraph (Micromeritics SediGraph II 5100).

Total concentrations of Cd, Cr, Cu, Ni and Pb were measured in oven-dried (75 °C) soil samples, ground into a fine powder by an agate mortar (Fritsch Analysette Spartan 3 Pulverisette 0) and digested by HF (50%) and HNO_3 (65%) at a ratio of 1:2 (v:v) in a micro-wave oven (Milestone-Digestion/Drying Module mls 1200).

The available metal fractions were extracted from the oven-dried samples by the method of Lindsay and Norwell (1978) for soils with pH > 6.5 (diethylenetriamine pentacetic acid [DTPA], $CaCl_2$ and triethanolamine [TEA] at pH 7.3), and by the method of Lakanen and Erviö (1971) for soils with pH < 6.5 (ethylendiaminetetracetic acid [EDTA] and ammonium acetate at pH 4.7).

The concentrations of Cd, Cr, Cu, Ni and Pb in digests and extracts were measured by graphite furnace atomic absorption spectrometry (Spectr AA 220 FS – Varian). Accuracy was checked by concurrent analysis of standard reference material from the Community Bureau of Reference of the Commission of the European Communities (BCR N° 142R, sandy loam soil): the results showed