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The use of Collembola avoidance tests to characterize sewage sludges as soil amendments

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A B S T R A C T

The ecotoxicological characterization of sewage sludge takes into account the additive, antagonistic and synergistic effects that occur as a result of multi-chemical interactions. Such an evaluation therefore is essential to complement the chemical analysis that, although required by law, is clearly insufficient. Using a tiered approach in the toxic evaluation of sewage sludge allows for characterization of toxicity in a timely manner. According to the literature, reproduction tests with *Folsomia candida* are suitable tools for the toxic assessment of organic sludges. Therefore, the inclusion of Collembola avoidance tests at a screening level (low tier), and acting as a trigger for longer-period tests (high tier; e.g. reproduction test), may provide a successful strategy, and may complement the currently proposed test battery. To evaluate the use of both avoidance and reproduction tests with collembolans in such a tiered approach, three sewage sludges (urban, olive and electroplating industries) were mixed in with a field-collected soil at different concentrations. Avoidance and reproduction tests were performed with the soil–sludge mixtures after 0, 4 and 12 weeks of incubation. The tests detected no toxicity in soil–sludge mixtures of urban and olive sludges at any incubation period. Mixtures with sludge from the electroplating industry induced toxicity only in the avoidance tests with freshly prepared and 4-week incubated samples. These results demonstrate the ability of Collembola avoidance tests to assess sewage sludge toxicity over time and its potential for hazardous sludge characterization at low tier levels.

1. Introduction

The growth of the World's industry has contributed to the increase of waste production. The European Community generates approximately two billion tons of waste per year (Düring and Gäth, 2002). A larger part of these wastes consists of industrial sludges that can constitute valuable soil amendments due to their high organic matter and nutrient contents (Phillips et al., 1997). Therefore, the land application of sewage sludge may be an acceptable form of waste disposal. However, the usual presence of contaminants in sludge (e.g. metals) can provoke adverse effects on the environment (Düring and Gäth, 2002).

The ability to evaluate the potential eco(toxico)logical risk associated with sludge application is an important tool to prevent the contamination of soil ecosystems. The use of ecotoxicological tests to evaluate the toxicity of waste materials has gained strength with the European Council Directive 91/689/EEC (European Community, 1991), which includes the "Ecotoxic" property as one of the 14 criteria that should be considered when characterizing wastes as hazardous to the environment. The information obtained from chemical analysis is often insufficient because several relevant and persistent pollutants are not included and the interaction between chemicals is not considered. The contaminants included in a sewage sludge can cause additive, antagonistic and synergistic effects resulting from multi-contaminant interactions. These phenomena are tightly dependent on the bioavailable fraction and have to be taken into account to obtain a valuable hazardous waste evaluation. Bio-assays have the potential to evaluate the toxicity of a waste as a whole and therefore work as a complement to chemical analysis (Wilke et al., 2008).

There is still no consensus in the scientific community about the battery of ecotoxicological tests that should be adopted for hazardous waste characterization. Recently, studies have been conducted to evaluate the usefulness and suitability of standardized test batteries aimed at waste characterization (Pandard et al., 2006;

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Alvarenga et al., 2007; Rosa et al., 2007; Wilke et al., 2008; Natal-da-Luz et al., 2009; Moser and Römbke, 2009). The use of soil invertebrates as test organisms in ecotoxicological testing has been shown to be suitable for the evaluation of the toxicity of certain contaminants present in sewage sludges, such as linear alkylbenzene sulphonates (Krogh et al., 2007).

Earthworms comprise the bulk of soil invertebrates mostly used in sludge toxicity evaluations (Pandard et al., 2006; Krogh et al., 2007; Alvarenga et al., 2007). However, the inclusion of other invertebrate test species, with different sensitivities and alternate route(s) of exposure, for this type of evaluation would improve the relevance of test results and provide a useful addition to a standardized test battery. Considering the impact on microbial communities following the application of sewage sludge to agricultural soil, it is predictable that microbivorous organisms like collembolans, which are dependent on fungi and bacteria populations, are more sensitive and, consequently more suitable, as a bio-indicator of bio-solid toxicity in soil (Cole et al., 2001). Moreover, earthworms are more influenced by organic matter (OM) content in the soil than Collembola (Natal-da-Luz et al., 2008a). This leads us to hypothesize that the sensitivity of earthworms can be lower than that of collembolans, due to the presence of a higher OM content in sludge-treated soils that can mask the effect of contaminants.

Crouau et al. (2002) and Domene et al. (2007) showed that the collembolan species *Folsomia candida* has potential to be used in the ecotoxicological assessment of organic wastes. *F. candida* reproduction is a more sensitive endpoint than mortality (Krogh and Petersen, 1995) but it takes more time to assess. Therefore, in order to reduce the time needed for hazardous waste characterizations, it is critical to use a tiered assessment strategy comprising a screening (lower tier) and a more detailed phase (higher tier) (Moser and Römbke, 2009). In a tiered approach, reproduction tests are considered as higher tier levels but are only triggered when impairment is detected with lower tier tests. This procedure prevents the use of certain long-term tests (e.g. with sludges that did not reveal toxicity at a screening level) without compromising the quality of the assessment.

Avoidance tests using soil invertebrates are rapid tests that can be used as lower tier tools in such an assessment scheme. In fact, earthworm avoidance tests are even included in the screening level of a proposed extended limit test design for ecotoxicological waste characterization (Moser and Römbke, 2009). Avoidance tests with *F. candida* are emerging and have been optimized using reference chemicals (Natal-da-Luz et al., 2008a, 2008b) and can be used instead of or complementary to earthworms. An ISO draft for avoidance tests with *F. candida* is currently being prepared (ISO, 2007b) in addition to the ISO draft for avoidance tests with earthworms (ISO, 2007a). These bio-assays, besides being fast and of low cost, already showed the potential to work as an early screening tool in risk assessment (Natal-da-Luz et al., 2004) and, more recently, in sludge characterization (Moreira et al., 2008; Natal-da-Luz et al., 2009). Avoidance tests with collembolans were also used to evaluate the microbial degradation of polycyclic aromatic hydrocarbons (PAHs) over time by Lors et al. (2006). However, the usefulness of this test type to evaluate sludge toxicity over time after its incorporation in soil has not been studied.

To fill this knowledge gap, avoidance and reproduction tests with *F. candida* were performed using concentration gradients in soil of three sewage sludges from three distinct sources. The effect of ageing was also investigated over a 12-week incubation period. The objectives of the present study were: (i) to assess the use of *F. candida* avoidance behavior and reproduction as ecotoxicological endpoints; and (ii) to evaluate the potential of using *F. candida* avoidance tests to assess the toxicity of sludge over time.

2. Materials and methods

2.1. Control soil

A loamy sand field soil from Central Portugal, collected in the sub-urban limits of the city of Coimbra, was used as a control soil. The soil was free of pesticide and fertilizer application for more than 5 years. The soil was sieved (mesh 5 mm) and defaunated through two freeze–thawing cycles (48 h at -20 °C followed by 48 h at 25 °C). The soil microbial community was re-established by inoculating the bulk soil with an elutriate obtained from a fresh soil sample (1:10 fresh soil:distilled water (w:w) mixed for 30 min). The parameters measured were soil pH (1 M KCl 1:6 v:v), water holding capacity (WHC; ISO, 1999), cation exchange capacity (ISO, 1994), organic matter content (OM; loss on ignition at 500 °C for 6 h), soil texture (LNEC, 1970) and total metal concentration (see below).

2.2. Test sludges

Three sewage sludges were obtained from distinct sources, containing different levels of metals, organic matter and pH (Table 1). Sludge A was obtained from a municipal wastewater treatment plant in Coimbra, Portugal; Sludge B from a biological and secondary treatment of wastewater from an olive processing industry (Mira, Portugal); and Sludge C from a biological and secondary wastewater treatment of sewage from an electroplating industry (Ceira, Portugal).

2.3. Treatments

The control soil was mixed with the test sludges in different proportions to obtain a concentration gradient for each test sludge equivalent to dosages of 0, 6, 15, 25 and 45 *t* dry weight (DW)/ha (which represent 0, 4, 10, 16.7 and 30 g DW/kg, respectively). These dosages are in agreement with the typical applications used in fertilization assays, taking into account the allowed legal limits (European Community, 1986). Sludge mixtures were prepared assuming a density of 1.5 g cm^{-3} for the control soil and that the test sludges would be incorporated to a depth of 10 cm. Once prepared, pH (1 M KCl 1:6 v:v), WHC (ISO, 1999), and OM content (loss on ignition at 500 °C for 6 h) of each mixture were determined. Each soil–sludge mixture was moistened to 50% of its respective WHC and incubated in a plastic box (36 cm long, 22 cm wide and 11 cm high) at a temperature of 20 ± 2 °C and a photoperiod of 16:8 h light:dark. Samples were collected from the incubation con-

Table 1
Total metal concentrations, pH and organic matter content of the test sludges (average ± standard deviation; *n* = 3) and the upper limit values of metals allowed for a sludge to be incorporated in agricultural soil according to Directive 86/278/CEE of the European Community (1986). ND – not determined; QL – not detected or present at a concentration below limit of quantification; A – urban sludge; B – sludge from olive processing industry; C – sludge from electroplating industry.

Sludge	A	B	C	Limit values
pH (1 M KCl)	6.57 ± 0.13	7.68 ± 0.08	8.57 ± 0.04	–
Organic matter (%)	74.9 ± 2.1	64.6 ± 4.1	4.4 ± 1.1	–
Metals in bulk sludge (mg kg^{-1})				
Cadmium	3.2	<0.5	<QL	20
Chromium	121	74	4790[a]	1000
Copper	436	66	42	1000
Lead	145	19	3.5	750
Mercury	ND	0.3	0.07	16
Nickel	39	33	58	300
Zinc	1731	350	900	2500

[a] 3720 mg Cr(III) kg^{-1} and 1070 mg Cr(VI) kg^{-1}.