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Ecotoxicological assessment of organic wastes using the soil collembolan *Folsomia candida*

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Abstract

The reproduction test with the collembolan *Folsomia candida* is used as a tool to evaluate the ecotoxicological potential of organic wastes currently applied to soil. Seven organic wastes (dewatered sewage sludges, thermally dried sewage sludges, composted sewage sludges, and a thermally dried pig slurry) were tested. These wastes had different origins, treatments, and pollutant burdens, and were selected as a representative sample of the wide variety of wastes currently generated. *F. candida* showed varied sensitivity depending on the waste, but also depending on the endpoint assessed. Reproduction was more sensitive than survival, although no correlations between reproduction and physico-chemical parameters and pollutant burden could be found. On the other hand, mortality was directly related to the lack of stability of wastes, probably reflecting the toxicity of end-products such as ammonium. Body length was not shown to be a sensitive endpoint for waste testing, as it was neither affected nor even stimulated by waste concentrations.

Organic matter, pH, and electrical conductivity varied with waste concentration in soil-waste mixtures, although their effect on collembolan performance was expected to be low and part of the complex effect exerted by wastes when applied to real soils. Selection of the water content is the most problematic aspect in waste testing, as it may affect the performance of test organisms. In this study, a qualitative approach for water content selection in waste testing was considered to be the most suitable.

Treatment of wastes affected composition and toxicity. Composting of sewage sludge increased its stability, compared to the initial sludge, but decreased its non-persistent organic pollutant burden and toxicity. On the other hand, thermally dried wastes from sludge and pig slurry displayed high toxicity, mainly attributable to their low stability. The results from the study indicate the inability of chemical methods to predict the effects of complex mixtures on living organisms with respect to ecotoxicity bioassays, but also the need for stabilization treatments of organic wastes prior to their reuse in soils.

Keywords: *Folsomia candida*; Survival; Reproduction; Body length; Organic wastes; Stability

1. Introduction

The amount of sewage sludge produced in the European Union has increased dramatically in recent years due to the implementation of Directive 91/271/EC. This increase will mainly be managed through its reuse in agricultural soil, despite our poor understanding of the impact of this management option. There is a large amount of experimental evidence, which suggests that this practice may enhance soil fertility, but there are also well-known associated environmental risks, including pathogens, nitrate pollution of ground waters, and inputs

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of heavy metals and organic pollutants (Düring and Gath, 2002).

To date, experimental results of sludge application to agricultural soils indicate a low level of risk for crops, but little is known about its effects on soil biota, a critical element in soil functioning (Giller et al., 1997). Harmful effects on soil invertebrates have been found in laboratory experiments (Krogh et al., 1997; Andrés and Domene, 2005), but some field experiments have shown that soil biota are stimulated when sludge is added to soil at agronomic rates (Krogh and Pedersen, 1997; Petersen et al., 2003).

Measuring pollutant concentration by chemical methods is the most common way to estimate the toxicity of pollutants and wastes, despite the development of biological methods in recent decades and their advantages over chemical methods. For example, the European Union regulation restricts the reuse of sewage sludge in soil taking into account limit values for six heavy metals (Directive 86/278/EC), but no biological tests are mentioned, even in the third draft of the Working Document on Sludge (European Communities, 2000). Furthermore, methods to assess the direct toxicity of solid wastes are not available despite the existence of standardized protocols for single chemicals using terrestrial organisms.

Crouau et al. (2002) concluded that the standardized Collembola reproduction test ISO 11267 (1999) was suitable for this purpose. They also pointed out that reproduction in this species may be affected not only by pollutant content but also by physico-chemical characteristics of waste such as pH, moisture and organic matter content. As a result, bioassays applied to organic wastes were not easy to interpret as two contradictory effects occurred at the same time. On the one hand, the organic matter in residues may have a stimulatory effect on soil organisms, while on the other hand the pollutant burden may exert inhibitory effects (Krogh et al., 1997; Andrés and Domene, 2005). Furthermore, parameters such as water availability or pH may also contribute to the biological effects observed.

The main aim of this study was to assess the suitability of the *Folsomia candida* reproduction test as a tool for the ecotoxicological assessment of organic wastes, which are to be applied to soils. Special attention was devoted to the special characteristics of waste testing, which involves variation in the physico-chemical properties of the soil-waste mixtures as the waste concentration increases. In addition, the influence of the origin, treatment, and composition of organic wastes on the ecotoxicological response of *F. candida* were be studied.

2. Methods

2.1. Test species

The strain of *F. candida* used in our experiments was provided by the Institute of Ecological Science of the Free University of Amsterdam. Cultures were raised in polyethylene containers 17.5 cm × 12.5 cm × 7.5 cm. The substrate consisted of a 1 cm layer of a wet mixture of plaster of Paris and charcoal (9:1, v/v). Cultures were raised in darkness in a climatic chamber at a constant temperature of 21 ± 1 °C. The substrate was renewed and the density of individuals was reduced every 2 months to avoid overcrowding.

2.2. Organic wastes

In order to represent a variety of organic wastes currently applied to agricultural soils, we selected seven types of waste: two dewatered sewage sludges, two composted sewage sludges, two thermally dried sewage sludges, and a thermally dried pig slurry. Treatments and post-treatments of the wastes differed as summarized in Table 1.

Physico-chemical properties, heavy metal and organic pollutant contents of the wastes are recorded in Table 2. Dry matter, water holding capacity, water pH, electrical conductivity, total nitrogen, and organic matter were measured according to EN 12880 (2000), ISO 11267 (1999), EN 13037 (1999), EN 13038 (1999), EN 13342 (2000) and EN 12879 (2000), respectively.

Non-hydrolyzable (stable) organic matter and non-hydrolyzable nitrogen were measured as a percentage of organic matter and nitrogen remaining in the sample residue after acid hydrolysis, as described in Rovira and Vallejo (2002). This method removes the more labile fraction of an organic substrate, mainly consisting of polysaccharides and proteins. Hydrolyzable nitrogen was calculated by subtracting the content of non-hydrolyzable nitrogen from total nitrogen content. N-NH_4 was measured on the distillates obtained from fresh samples.

Elemental analysis of P, K, Cd, Cr, Cu, Hg, Ni, Pb and Zn was carried out by ICP-MS according to ISO 11885 (1996). Polychlorinated dibenzodioxins and dibenzofuranes (PCDD/F) were measured with HRGC-HRMS, polychlorinated biphenyls (PCB) by HRGC-ECD, di(2-ethylhexyl)phthalate (DEHP) and nonylphenols (NPE) by HRGC-MS. Polycyclic aromatic hydrocarbons (PAH) and linear alkylbenzene sulphonates (LAS) were determined by HPLC with fluorescence and UV detectors, respectively. Values for each pollutant group