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Organic wastes as soil amendments – Effects assessment towards soil invertebrates

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HIGHLIGHTS

- Chemical analyses may be not sufficient to assess risk of organic wastes.
- A bioassay test battery is proposed to complement chemical analyses.
- Chemical analyses complemented with bioassays support ERA of wastes.
- Chemical and biological data provide information on sources of waste toxicity.

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ABSTRACT

Using organic wastes, as soil amendments, is an important alternative to landfilling with benefits to soil structure, water retention, soil nutrient and organic matter concentrations. However, this practice should be monitored for its environmental risk due to the frequent presence, of noxious substances to soil organisms. To evaluate the potential of eight organic wastes with different origins, as soil amendments, reproduction tests with four soil invertebrate species (*Folsomia candida*, *Enchytraeus crypticus*, *Hypoaspis aculeifer*, *Eisenia fetida*) were performed using gradients of soil–waste mixtures.

Results obtained demonstrated that contaminant concentrations required by current legislation might not be a protective measure for the soil ecosystem, as they do not properly translate the potential toxicity of wastes to soil invertebrates. Some wastes with contaminant loadings below thresholds showed higher toxicity than wastes with contaminants concentrations above legal limits. Also, test organism reproduction was differently sensitive to the selected wastes, which highlights the need to account for different organism sensitivities and routes of exposure when evaluating the toxicity of such complex mixtures. Finally this study shows that when combining chemical and ecotoxicological data, it is possible to postulate on potential sources of toxicity, contributing to better waste management practices and safer soil organic amendment products.

1. Introduction

Waste production and management is a global concern that will tend to worsen with the increase of the world's population. As an example, in 2010 the European Union produced 2.5 billion tons of waste, over 900 million tons of which was either landfilled or burnt. However, more than 50% of these residues could have been either re-cycled or re-used [1]. For instance, the land application of wastes in agricultural fields could be an important recycling alternative aiming to reduce landfilling. The application of organic wastes as soil amendments has positive effects on soil organic matter (OM) content, macro and micro-nutrients, reducing the need of inorganic fertilizers [2,3]. Furthermore, the use of organic wastes in soil can aid in the formation of soil macro-aggregates, increasing soil structure [4], water infiltration and soil water-holding capacity [5]. Thus, the proper management of organic wastes as soil amendments can improve the essential goods and services provided by soil such as water filtration, food production

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and climate regulation [6,7]. The advantages of organic amends are very important for Mediterranean soils which have naturally low OM content and are prone to erosion.

Despite these potential benefits, wastes are complex mixtures composed of several constituents (some of them unknown) from different sources and can contain hazardous substances affecting soil functioning and the provisioning of soil ecosystem services essential for crop land productivity [8]. Therefore, the application of wastes to agricultural land has been controlled through legislative measures. Since 1986, sewage sludge application has been legislated by European Directive 86/278/EEC which is currently under revision [9,10] and has been updated in Portugal through Decree-Law No. 276-2009 to include limits for organic contaminants and pathogenic micro-organisms (*Escherichia coli* and *Salmonella* spp.) [11]. For other organic wastes, such as composts, their application to soil has no current legislation in the European Union, but measures have been proposed in a waste criterion report [12]. In Portugal, compost application has recently been legislated by the fertilizer materials application Decree-Law No. 103/2015 [13].

The limits established by law for the application of organic wastes to soil (including sludges and composts) consider solely chemical parameters. Although chemical parameters are extremely important to characterize wastes and the potential sources of pollution, they have known limitations, namely the number of compounds that are usually screened and the lack of predicting effects due to the interactions between them. On the other hand, ecotoxicological tests, either single species or community-based assays, by exposing the organisms to the wastes, can effectively assess the hazard of these complex matrices. The combination of both chemical and ecotoxicological parameters allows a higher success when attempting to protect the soil compartment from the potentially hazardous effects arising from the application of wastes to soil.

The use of soil invertebrates in laboratory tests to assess the toxicity of wastes has been proposed by several authors [14–25]. Up to now, the produced literature focuses on either only a single bioassay endpoint or considers a test battery where other organisms, such as aquatic invertebrates (i.e. *Daphnia magna*), bacteria (i.e. *Vibrio fischeri*), microalgae (i.e. *Scenedesmus subspicatus*) or plants may also be considered, which leads to only one or two species of soil invertebrates being included [14,19,23,24]. Also, these studies generally test a reduced number of wastes, often with similar origin [17,21,22]. The definition of a suitable test battery to assess the ecotoxicity of hazardous wastes has been discussed by Wilke et al. [23] and Kapanen and Itävaara [26] but specifically for compost application.

In this paper, the effect of organic wastes on the reproduction of soil invertebrates was determined for eight different wastes of contrasting origin using four soil invertebrate species. The toxicity of the wastes and their chemical properties were discussed along with the difference in sensitivity of the test organisms in an attempt to define which could be the most suitable species to assess the risk to soil invertebrates according to waste contamination profile. Results obtained represent the first assessment tier of a larger project (ResOrgRisk–PTDC/AAC-AMB/119273/2010 funded by FCT – Fundação para a Ciência e a Tecnologia) that aimed to assess the potential risk of land application of organic wastes as soil amendments.

2. Materials and methods

2.1. Soil

A natural soil collected from the top 20 cm layer of an agricultural field from the Polytechnic Institute of Beja, located in the sub-urban limit of the city of Beja, South of Portugal, was used in laboratory tests. This soil was sieved at 5 mm and defaunated by two freeze and thaw cycles (48 h at −20 °C followed by 48 h at 25 °C). Soil microbial communities were re-established after defaunation by adding 5 ml (per kg of soil) of soil elutriate produced with fresh non-defaunated soil (1:10, soil:water proportion, w:w, mixed for 30 min). Standard artificial soil was also used as a control to validate experimental procedures. This soil was prepared by mixing sand, kaolinite clay and *Sphagnum* sp. peat in a proportion of 75:20:5 (w:w:w), respectively, and $CaCO_3$ was added to adjust the pH to 6 ± 0.5.

2.2. Wastes

A total of eight wastes selected from several different industrial and agricultural activities and in different forms (three sludges, three composts, one digestate and one sludge + ashes mixture) were used in laboratory tests. The full list of wastes with information about their origin and usual destination is presented in Table 1. Physical and chemical properties of wastes are presented in Table 2 and respective total metal and organic contaminants concentrations are presented in Table 3. For detailed information on methodological procedures adopted in these measurements see Alvarenga et al. [27], which reports the full physical and chemical properties of the test wastes used in the present study. All wastes were transported from their production facilities and used directly in the laboratory tests except sewage sludges (SS1 and SS2), which were defaunated and re-inoculated (as described above for the natural soil) before the experiments to eliminate diptera larvae. For reproduction tests, each waste was mixed with the natural soil in different proportions (% of waste DW in the waste–soil mixture) creating a gradient to allow the estimation of an EC_{50} value for each test species. Pictures of each waste at the time of testing are provided in supplementary data (Fig. 1SD).

2.3. Test organisms and culture conditions

All test species were maintained in a temperature controlled chamber at 20 ± 2 °C under a photoperiod of 16 h light and 8 h dark.

Folsomia candida Willem (Arthropoda, Hexapoda, Collembola, Isotomidae) cultures were maintained in cylindrical transparent plastic boxes (11 cm diameter and 4 cm height) with the bottom filled with a mixture of plaster of Paris and activated charcoal in a proportion of 11:1 (w:w). Springtails were fed twice a week with dry and granulated yeast and mouldy food was removed from the culture medium when observed. The moisture of the culture medium was maintained with distilled water weekly. For laboratory tests, 10–12 day old individuals were used from synchronized cultures.

Enchytraeus crypticus Westheide & Graefe (Annelida, Clitellata, Oligochaeta, Enchytraeidae) cultures were maintained in petri dishes with agar medium as described by Cesar et al. [28]. Cultures were fed weekly with finely ground rolled and previously autoclaved oats. For testing, organisms with a visible well-developed clitellum were selected.

Eisenia foetida Savigny (Annelida, Clitellata, Oligochaeta, Lumbricidae) cultures were maintained in plastic boxes with a substrate composed of horse manure and *Sphagnum* sp. peat (1:1, w:w) and a small amount of $CaCO_3$ to adjust the pH. Cultures were fed with horse manure and moisture was adjusted weekly. Earthworms with a well-developed clitellum, more than one month old and with an average weight of 264.3 ± 32.0 mg (average ± SD, n = 1800) were used for testing.

Hypoaspis aculeifer Canestrini (Arthropoda, Arachnida, Mesostigmata, Laelopidae) cultures were kept in cylindrical plastic boxes (11 cm diameter and 4 cm height) with the bottom filled with a mixture of plaster of Paris and activated charcoal in a proportion of 9:1 (w:w). Individuals were fed with cheese mites