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Ecotoxicological assessment of organic wastes spread on land: Towards a proposal of a suitable test battery

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ABSTRACT

The land spreading of organic wastes in agriculture is a common practice in Europe, under the regulation of the Directive 86/278/EEC. One of the objectives of this Directive is to prevent harmful effects of organic wastes on soil, plants and animals. Despite this regulatory framework, there is still a lack of harmonized ecotoxicological test strategy to assess the environmental hazard of such wastes.

The aim of this study was to provide a first step towards the *a priori* ecotoxicological assessment of organic wastes before their land use. For that purpose, nine different organic wastes were assessed using direct (i.e. terrestrial tests) and indirect (i.e. tests on water eluates) approaches, for a total of thirteen endpoints. Then, multivariate analyzes were used to discriminate the most relevant test strategy, among the application rates and bioassays used.

From our results, a draft of test strategy was proposed, using terrestrial bioassays (i.e. earthworms and plants) and a concentration range between one and ten times the recommended application rates of organic wastes.

1. Introduction

The implementation of the European Directive 86/278/EEC (EC, 1986) and of the Directive 91/271/EEC (EC, 1991a), respectively on the use of sewage sludge in agriculture and on urban wastewater treatment, has led to an increase in the production and recycling of organic wastes as fertilizers or soil amendments in Europe. With a production of raw materials of approximately 250 million tons of municipal wastes per year in the EU, and more than 850 million tons of industrial wastes (SOER, 2010), the recycling of organic wastes in agriculture has now become a common practice, and thus a major issue for both producers and farmers. To comply with sustainable practices, stakeholders are proposing a wide variety of organic wastes for agricultural use. For example, in France, the National Agency for Food Environmental and Occupational Health & Safety (ANSES) already approved the agricultural recycling of organic wastes (individually or mixed) such as sewage sludge, ashes, composted wastes, sawdust, household wastes, industrial residues, animal feces, or more specific residues, like homogenate of eggshell, shellfish wastes or extinguisher powder mixed with beet wastes (http://e-phy.agriculture.gouv.fr/).

As such wastes are a potential source of organic matter and fertilizing compounds, e.g. nitrogen, phosphorus and/or potassium, they can be used to improve soil characteristics and crop production. However, regarding their origin and treatments, organic wastes can also be a potential source of pollutants affecting soil biota (Düring and Gäth, 2002) and water resources quality (Smith et al., 1999). Potential contaminants that may be found in these materials ranged from heavy metals to persistent organic compounds, including emerging contaminants such as pharmaceuticals and endocrine disruptors (Eriksson et al., 2008; Clarke and Smith, 2011), as well as nanoparticles and/or their derivatives (Kim et al., 2010; Lombi et al., 2012). Therefore, the land use of these wastes is raising concern considering soil protection (EC, 2002), and also because it is essential to demonstrate their innocuousness towards human being and the environment before their land use.

The adverse effects of organic wastes on the environment and especially the effects on living organisms (i.e. micro-organisms, plants and invertebrates) are still under-represented in the regulatory assessment of these complex matrices. This is due to the fact that the environmental assessment of these materials is mainly based on their physico-chemical characteristics. For example, heavy metals contents must comply with defined thresholds as raw materials, and also with heavy metals thresholds in the soil to which it is applied. To date, the ecotoxicological assessment

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of organic wastes is not mandatory before their approval. Nevertheless, potential priority organic pollutants were highlighted in sewage sludge (Eriksson et al., 2008; Clarke and Smith, 2011), but it is not reasonably achievable to include all of them in European regulations. The physico-chemical characterization cannot thus be considered sufficient alone for the determination of the potential ecotoxicity of these materials, as claimed by several authors (Kapanen and Itävaara, 2001; Alvarenga et al., 2007; Natal–da–Luz et al., 2009, Delgado et al., 2012).

Over the last years, there were many attempts to determine the ecotoxicity of various organic wastes, from sewage sludge to ashes, also including different composted materials and/or animal feces. Due to the non-harmonization of the environmental assessment of such kind of materials, the available studies and produced data are heterogeneous, from field studies (Axelsen and Kristensen, 2000; Cole et al., 2001) or semi-field studies (Andrés and Domene, 2005; Carbonell et al., 2009; Delgado et al., 2012) to laboratory studies, including tests on bacteria (Mantis et al., 2005), on terrestrial organisms or terrestrial plants (Gunadi and Edwards, 2003; Oleszczuk, 2008; Ramírez et al., 2008) as well as tests battery approaches (Selivanovskaya and Latypova, 2003; Bostan et al., 2005; Alvarenga et al., 2007; Chiochetta et al., 2014).

Among European studies aiming at assessing the ecotoxicity of organic wastes before their land use, two complementary approaches are usually applied: a direct approach, i.e. solid-phase tests, and an indirect one, i.e. liquid-phase tests, assessing extracts of the samples. For terrestrial approaches (Alvarenga et al., 2007; Domene et al., 2008; Moreira et al., 2008; Oleszczuk, 2008; Ramírez et al., 2008), methodologies differ according to the soil used, i.e. natural and/or artificial, and according to the metrics used to express the results, i.e. as application rates (t/ha) or in mass ratio (g/kg or as a % of the organic waste mixed with soil). For liquid-phase approaches, the main differences concern the extraction procedures performed, i.e. eluates of mixtures from microcosms (Delgado et al., 2013), different water and solvent extracts (Domene et al., 2008) or water extracts using standard protocols (Alvarenga et al., 2007; Lapa et al., 2007; Malara and Oleszczuk, 2013; Mantis et al., 2005). In these standards (DIN 38414-S4, 1984; CEN, 2002), eluates are obtained from mixtures prepared in water using a ratio of 10 L/kg, followed by agitation and centrifugation. However, for all of these studies, the ecotoxicity in liquid-phase was expressed using different percentages of eluates diluted in bioassay media, in order to calculate LC50 and/or EC50 values.

Bioassays used in the above-mentioned studies were mainly *in vivo*, using higher plants, earthworms, collembolan for solid-phase tests; and bacteria, algae, crustacean for liquid-phase tests. Some authors also proposed *in vitro* bioassays, assessing the cytotoxicity on fish cell lines (Delgado et al., 2013). To date, no test strategy was however developed for the ecotoxicity assessment of organic wastes recycled in agriculture. Most efforts focused on propositions of test strategies to determine effluent quality (Manusadžianas et al., 2003; Hernando et al., 2005; Mendonça et al., 2009; Tigini et al., 2011), soil quality (Bierkens et al., 1998; Van Gestel et al., 2001; Achazi, 2002; Eisentraeger et al., 2004), sediment quality (Ahlf et al., 2002; Ahlf and Heise, 2005; Höss et al., 2010; Tuikka et al., 2011) and the HP14 (ecotoxic) property of wastes included in the European Directive 2008/98/EC (EC, 2008) (repealing Directive 91/689/EEC (EC, 1991b)) for waste classification (Pandard et al., 2006; Wilke et al., 2008; Pablos et al., 2009; Stiernstroem et al., 2009, Weltens et al., 2012; Pandard and Römbke, 2013). But some authors stressed the need of a specific ecotoxicological assessment for organic wastes before their spreading (Domene et al., 2008; Natal–da–Luz et al., 2009). For that purpose, these authors assessed the suitability of different terrestrial and aquatic bioassays test batteries for the evaluation of organic wastes. These test batteries were built up to allow a tiered approach assessment, e.g. organic waste toxicity screening using behavioral responses, followed by acute and chronic toxicity tests with different endpoints. Furthermore, species used in the above-mentioned studies were selected to be representative of the soil and aquatic communities, as well as of producers (bacteria, algae, plants), primary consumers (crustacean, collembolan) and decomposers (earthworms).

The aims of the present study were thus (i) to provide a first step towards the *a priori* ecotoxicological assessment of organic wastes before their land use, and (ii) to propose an evaluation strategy combining the most relevant *in vivo* bioassays, selected from batteries of solid-phase and liquid-phase tests. For that purpose, the potential ecotoxicity of nine organic wastes were assessed in mixtures with artificial soil (and natural soil for nematode tests), which is representative of their environmental use and also permit to comply with standard procedures. Test organisms used for the direct approach (plants, earthworms, nematodes) and the indirect approach (bacteria, algae, crustacean, rotifer, nematodes) were chosen as they were complementary in term of trophic level (i.e. producers, primary consumers and decomposers) as well as in term of studied endpoints (i.e. behavioral, acute and chronic endpoints). The ecotoxicity of the studied organic wastes was determined using different spreading schemes. This test strategy includes both the respective recommended application rates of each material and multiples of these application rates (five, ten, fifty and one hundred times). The last two were added to determine the limitations of the selected bioassays with high application rates and to set up a first draft of biological responses database for such materials. Results of the bioassays, which represent a total of thirteen endpoints, were then used to perform multivariate analyzes in order to select the most relevant and informative endpoints to characterize the hazard of the organic wastes studied.

2. Materials and methods

2.1. Origin, treatment and preparation of the organic wastes

Nine different organic wastes were considered, i.e. six industrial and urban wastes, two composted materials and one animal feces (photos in Supplementary data 1). These materials are representative of commonly used organic wastes in Europe and some were already approved for land spreading. Their origin, treatment and application rate are summarized in Table 1. After reception, these organic wastes were stored at 4 °C. Most of them were used without any pre-treatment, excluding the manure which was grinded and sieved (< 4 mm), and the two composted materials which were only sieved (< 4 mm) before testing.

2.2. Characterization of the organic wastes

The fertilizing and physico-chemical characteristics of the organic wastes are listed in Supplementary data 2. Among the organic wastes, only the physico-chemical characteristics of the composted sewage sludge (CSS) were not provided and thus determined by an accredited laboratory (Laboratory of Soil Analyzes of the French National Institute for Agricultural Research (INRA), Arras, France). The water holding capacity and the dry matter content of the organic wastes were determined before testing, to be more accurate in the preparation of mixtures with artificial and natural soils.

2.3. Experimental plan and samples preparation

Terrestrial bioassays were performed on mixtures of organic