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Ecotoxicological characterization of sugarcane vinasses when applied to tropical soils☆

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HIGHLIGHTS

- In Brazil sugarcane vinasse is applied in agricultural soils to be used as fertilizer.
- Toxic potential of three vinasses was evaluated in different soils by standard tests.
- All tested vinasses showed sub-lethal effects on the test species.
- Vinasses derived from commercial distillery plants were the most toxic ones.
- Toxicity of vinasses varied with the application dose, test species and soil type.

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ABSTRACT

The impact of sugarcane vinasse on soil invertebrates was assessed through ecotoxicological assays. Increasing concentrations of two vinasses from different distillery plants (VA and VB), and a vinasse from a laboratory production (VC), were amended on two natural tropical Oxisols (LV and LVA) and a tropical artificial soil (TAS) to characterize the effects of the vinasses on earthworms (*Eisenia andrei*), enchytraeids (*Enchytraeus crypticus*), mites (*Hypoaspis aculeifer*) and collembolans (*Folsomia candida*). The highest concentrations of VA and VB were avoided by earthworms in all soils and by collembolans especially in the natural soils. The presence of VC in all of the tested soils did not cause avoidance behavior in these species. The reproduction of earthworms, enchytraeids and collembolans was decreased in the highest concentrations of VA and VB in the natural soils. In TAS, VB reduced the reproduction of all test species, whereas VA was toxic exclusively to *E. andrei* and *E. crypticus*. The vinasse VC only reduced the number of earthworms in TAS and enchytraeids in LVA. The reproduction of mites was reduced by VB in TAS. Vinasses from distillery plants were more toxic than the vinasse produced in laboratory. The vinasse toxicities were influenced by soil type, although this result was most likely because of the way the organisms are exposed to the contaminants in the soils. Toxicity was attributed to the vinasses' high salt content and especially the high potassium concentrations. Data obtained in this study highlights the potential risk of vinasse disposal on tropical soils to soil biota. The toxic values estimated are even more relevant when considering the usual continuous use of vinasses in crop productions.

1. Introduction

The search for energy technologies from renewable sources that have a low environmental impact, such as the production of biofuels, is a global challenge. Brazil is the only country that uses ethanol on a large scale as an alternative renewable fuel source to petroleum (Laime et al., 2011), and is currently one of the largest sugarcane ethanol producer with a production of approximately 23.6 billion liters in 2012/13 (MME, 2014). The production of this biofuel generates great volumes of vinasse, an acidic dark-brown organic wastewater with considerable amounts of organic matter (OM), potassium, and lower amounts of calcium and magnesium, among other substances (España-Gamboa et al., 2011). Each liter of ethanol produced generates about 8–15 l of vinasse (Freire and Cortez, 2000), which is mainly used in fertigation of agricultural soils (Laime et al., 2011; Christofoletti et al., 2013a).

Although fertigation with sugarcane vinasse can increase crop yields by acting as a water and nutrient source for plants and improving

☆ We declare that these experiments were conducted in accordance with EC Directive 86/609/EEC and national and institutional guidelines for the protection of human subjects and animal welfare.

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certain soil properties (Oliveira et al., 2009; Laime et al., 2011; Jiang et al., 2012; Christofoletti et al., 2013a), its environmental impacts have raised concerns because of the presence of contaminants (e.g. heavy metals, alcohol and phenolic compounds and antibiotic substances) in this type of wastewater (Almeida, 1952; Christofoletti et al., 2013a). When applied indiscriminately, vinasse may cause nutrient imbalance and salt saturation in the soil, leading to ion leaching to groundwater (Silva et al., 2007; Ribeiro et al., 2010). Furthermore, some studies have indicated that vinasse negatively affects physical soil properties, such as hydraulic conductivity and redox potential (Leal et al., 1983; Uyeda et al., 2013). In Brazil, the technical standard norm P4.231 (CETESB, 2006) establishes criteria and procedures for the application of sugarcane vinasse in agricultural soils. Aiming to prevent changes in the soil chemical properties (e.g. salt saturation), this standard has established that the maximum volume of vinasse for application should be estimated taking into account the cation-exchange capacity (CEC) of the soil, the soil potassium concentration in soil and vinasse and the amount of K_2O extracted by the sugarcane crop per hectare after each harvest (CETESB, 2006). Although these criteria are considered sufficient to comply with the objectives established in the standard norm, they do not consider the ecological effects derived from vinasse application, particularly those related to soil fauna and the associated ecological services.

The establishment of a strategy for waste application that does not compromise the presence of functionally stable communities of these organisms in the soil is of paramount importance, because the activity of soil invertebrates affects water infiltration and soil structure and has a key role in carbon, nitrogen, phosphorus and sulfur cycles through the fragmentation and decomposition of OM and regulation of microbial activity (Cortet et al., 1999; Brussaard et al., 2007; Cardoso et al., 2013). In the European Union, potential ecological risks of wastes should be assessed according the "Ecotoxic"-criterion H14, recently renamed HP 14 (Pandard and Römbke, 2013), from the European directive 2008/98/EC (that replaced the European Union Council Directive 91/689/EEC - EC, 1991, 2008). Laboratory ecotoxicological tests with standard species have been shown to be fundamental tools in the assessment of potential risks of wastes to the environment because these tests allow an integrated assessment of toxicity that considers the addictive, antagonistic and synergistic effects of contaminants. Additionally, the results obtained from ecotoxicological tests indicate the effect of the bioavailable fraction of contaminants in the waste; thus, they complement traditional chemical analyses (Pandard et al., 2006; Domene et al., 2007; Wilke et al., 2008; Natal-da-Luz et al., 2009a,b; Moser and Römbke, 2009; Cesar et al., 2014).

The toxicity of sugarcane vinasse for aquatic organisms is already known (Christofoletti et al., 2013a). However, only a very limited number of studies in terrestrial environments have evaluated the impact of this waste on soil fauna species. The presence of vinasse in soil is known to suppress certain nematode species (Pedrosa et al., 2005; Caixeta et al., 2011; Matos et al., 2011) and to cause mortality of diplopods (Christofoletti et al., 2013b). Therefore, we decided to partially fill this gap of knowledge and to contribute to the risk assessment of this residue, which is applied to tropical soils at an estimated rate of more than 200 billion liters/year (Laime et al., 2011; MME, 2014). This study aimed at characterizing the ecotoxicological potential of vinasse towards the invertebrate species *Eisenia andrei*, *Enchytraeus crypticus*, *Hypoaspis aculeifer* and *Folsomia candida*, standard species commonly used in ecotoxicological assessments. Vinasses from different origins were evaluated to determine their effects on these species on different soil types.

2. Materials and methods

2.1. Test soils and vinasses

Two Oxisols were used in the ecotoxicological tests. According to the Brazilian Soil Classification System: a Red Latosol (LV) with 33.6% of clay and a Red–Yellow Latosol (LVA) with 17.6% of clay. Both soils were collected at the 0–20 cm surface layer of sugarcane plantations in the state of São Paulo, Brazil. The sampling sites were free from application of sugarcane vinasses for more than 10 years. The soils were air-dried, sieved at 5 mm, and defaunated through three freeze-thawing cycles (48 h at −20 °C followed by 48 h at 25 °C) to eliminate the original soil fauna (Pesaro et al., 2003). Additional ecotoxicological tests were performed in tropical artificial soil (TAS) (Garcia, 2004). TAS is an adaptation of the Organisation for Economic Co-operation and Development (OECD) artificial soil (OECD, 1984) and is frequently used in laboratory ecotoxicological assays in tropical regions (Römbke et al., 2007; De Silva and Van Gestel, 2009; Alves et al., 2013, 2014). This soil consists of 70% sand (more than 50% of the particles sized between 0.05 and 0.2 mm), 20% kaolinite clay and 10% powdered coconut husks. When necessary, $CaCO_3$ was added to the mixture to obtain a TAS with a pH of 6 ± 0.5.

Physical and chemical characterization of the natural (LV and LVA) and artificial (TAS) soils was performed by measuring several parameters in the air-dried samples following the methodologies of Van Raij et al. (2001). The determination of available P, Ca, Mg and K was performed by the ion-exchange resin method. Available sulfur (S–SO_4) was extracted by the turbidimetric method in a 0.01 mol L^{-1} solution of Ca $(H_2PO_4)_2$. The elements Cu, Fe, Mn and Zn were extracted in diethylenetriaminepentaacetic acid–triethanolamine (DTPA–TEA; pH 7.3), whereas B was extracted in hot water/microwave. The exchangeable aluminum was determined by titration (1 mol L^{-1}), and potential acidity (H + Al) was determined by the Shoemaker–McLean–Pratt (SMP) buffer method. The soil OM was determined by colorimetry via oxidation with $Na_2Cr_2O_7.{}_2H_2O$ and H_2SO_4 (Van Raij et al., 2001). The maximum water-holding capacity (WHC) of the soils was determined according to the International Organization for Standardization (ISO, 1996), and the pH values were measured with a 1 M KCl solution (using a proportion soil:solution of 1:5, w/v). The sand, silt and clay fractions of the soils were evaluated by the pipette method, the clay level was determined with a Bouyoucos densimeter (Dane and Topp, 2002), and sediments were measured by subtracting the estimated sand and clay volumes from the total of the sample.

Three sugarcane vinasses with different origins were used in the ecotoxicological tests. Two vinasses were collected directly from tanks of different distilleries (VA and VB). As control, a third vinasse (VC) was produced in the laboratory and this did not include additional additives and antibiotics that are usually employed during the processes of fermentation and alcohol production (Compart et al., 2013). The physical and chemical characterization of the vinasses was performed according to the methods described by Kiehl (1985), which included measuring the following parameters: pH (1 M KCl), density, electric conductivity (EC), OM content (loss on ignition at 500 °C for 6 h), concentrations of mineral residues (soluble, insoluble and total), K (K_2O), Ca, Mg, S, total C levels (TC), N, P (P_2O_5), Cu, Mn, Zn, Fe and the C:N ratio.

The total concentrations of potentially toxic elements (PTE), including As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb and Zn, were determined for the soils and vinasses using extractions by microwave-assisted (CEM MARS Xpress™) digestion. Methods from the US Environmental Protection Agency were employed in the digestion of soils and vinasses, including EPA 3051A (0.5 g soil + 9 mL HNO_3 + 3 mL HCl) and EPA 3015A (45 mL vinasse + 4 mL HNO_3 + 1 mL HCl), respectively (USEPA, 1998, 1999). The PTE measurements in the soil and vinasse extracts were performed by the multi-element technique inductively coupled plasma optical emission spectrometry (ICP OES). The limits of quantification (LOQ) were 0.010, 0.002, 0.005, 0.005, 0.002, 0.010, 0.005, 0.001, 0.010, and 0.002 (mg L^{-1}) for As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb and Zn, respectively. The recovery of the extraction using the EPA 3051A method was verified by the inclusion of certified soil samples (Montana Soil, NIST, SRM 2711).