Ecotoxicology and Environmental Safety 147 (2018) 550–557

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Ecotoxicological and microbiological assessment of sewage sludge associated with sugarcane bagasse

Lais Roberta Deroldo Sommaggio[a], Dânia Elisa Christofoletti Mazzeo[b], Débora de Andrade e Silva Sant' Anna[c], Carlos Emílio Levy[c], Maria Aparecida Marin-Morales[a,*]

[a] Department of Biology, Institute of Biosciences, São Paulo State University (Unesp), Av. 24-A, 1515, 13506-900 Rio Claro, SP, Brazil
[b] Department of Analytical Chemistry, Institute of Chemistry, São Paulo State University (Unesp), Rua Professor Francisco Degni, 55, 14800-060, Araraquara, SP, Brazil
[c] Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Rua Alexander Fleming, 105, 13081-970 Campinas, SP, Brazil

ARTICLE INFO

Keywords:
Allium cepa
Chromosomal aberrations
Micronucleus
Microbiological characterization
Residue management

ABSTRACT

Sewage sludge (SS) obtained after sewage treatment process may contain several toxic substances. Bioremediation can decrease the toxicity of the sludge, mainly when it is associated with stimulant agents, such as sugarcane bagasse (B). Samples of pure SS (SSP); SS + B; SS + Soil; and SS + B + Soil were bioremediated for 1, 3, and 6 months (T1, T2, and T3, respectively). After each period, the cytotoxic, genotoxic, and mutagenic potentials of the solid samples and their respective aqueous extracts (aqueous eluate and percolate water) were evaluated by the *Allium cepa* test. A microbiological analysis of the samples was also performed after each period tested. All solid samples of SS + B (in T1, T2, and T3) and the solid sample of SSP (treatment T3) showed a significant decrease of cell division (cytotoxic effects). The aqueous eluate extracts of SS + B (T1 and T3) and SSP (T2 and T3) induced cytotoxic effect. The solid sample of SS + B (T2 and T3) and aqueous extracts of SSP (T1) were genotoxic, indicating a harmful effect of SS on *A. cepa*, even after 6 months of bioremediation. There was an alternation in the microbial community both in diversity and in abundance, with the predominance of non-fermenting gram-negative bacilli. The tested bioremediation periods were not sufficient for the complete detoxification of SS, and the use of B did not seem to contribute to the degradation of the pollutants to inert compounds. These data emphasize that a specific relationship should exist between the sludge characteristic and the biostimulating agent used to promote a more efficient bioremediation. These results suggest the necessity to study longer periods of biodegradation and the use of other decomposing agents for greater safety and sustainability for the agricultural use of this residue.

1. Introduction

Large amounts of sewage sludge (SS) are generated worldwide, creating an environmental contamination problem. The studies of Kelessidis and Stasinakis (2012) show that in European countries, such as Germany, Spain, France, and Italy, SS production is respectively 2.17; 1.121; 1.059, and 1.053 million tons of dry solids per year respectively. The studies of Pedroza et al. (2010) indicate the production of this residue in Brazil reaches around 150–220 thousand tons of dry matter/year. Wastes from wastewater treatment plants (WWTP) can be a global problem since the amount of waste generated is directly related to population density and water quality (Kliopova and Makarskienė, 2015) and, consequently, the socio-economic level of the country.

Some authors have noted that the composition of SS is related to the origin of the treated sewage, the type of treatment used by the WWTP, and the season of the year in which it was produced, so that this residue can present quite diverse characteristics (Bettiol and Camargo, 2006; Fytili and Zabaniotou, 2008; Silva et al., 2003; Singh and Agrawal, 2008). Rodríguez-Morgado et al. (2015) also highlighted that the use of different types of SS in the soil can generate distinct enzymatic activities due to the variation in the microbiological community present in each SS.

Due to the SS environmental risks, its growing world production and its rich composition of organic matter and nutrients (e.g., nitrogen, phosphorus, potassium, calcium, copper, cadmium, zinc, and manganese), some researchers have suggested the use of SS as an agricultural input, fertilizer or for reconditioning arable soil (Bovi et al., 2007; Singh and Agrawal, 2008; Mcgeehan, 2012; Gianico et al., 2013).

* Corresponding author.
E-mail addresses: rds.lais@gmail.com (L.R.D. Sommaggio), decm@rc.unesp.br (D.E.C. Mazzeo), debssantanna@gmail.com (D.d.A.e.S. Sant' Anna), celevy@fcm.unicamp.br (C.E. Levy), mamm@rc.unesp.br (M.A. Marin-Morales).

http://dx.doi.org/10.1016/j.ecoenv.2017.09.009
Received 13 May 2017; Received in revised form 1 September 2017; Accepted 5 September 2017
Available online 15 September 2017
0147-6513/

Nevertheless, SS can also contain harmful substances for living organisms and the environment, such as copper, nickel, lead, cadmium, and mercury, in addition to organic compounds, such as phenols, benzenes, anthracenes, linear sulfonated alkylbenzenes, and pathogenic microorganisms (Almeida et al., 1998; Holmstrup et al., 2001; Lopes et al., 2005; Lourenço, 1997; Paraiba and Saito, 2005).

Bioremediation is a widely used approach to reduce the toxicity of organic waste by the use of living organisms that are able to metabolize, transform or decompose pollutants so that toxic substances are converted into less harmful products (Bamfoth and Sigleton, 2005; Makadia et al., 2011). An employed strategy to assist this technology is biostimulation, which involves the addition of stimulating agents to increase the native microbiota growth. The porosity and soil aeration can be improved by physical means or by the addition of decompacting materials, which allow the entrance of air into the system and the consequent aerobic conditions in the process (Vasudevan and Rajaram, 2001), favoring the biostimulation process. Sugarcane bagasse is a good soil decompacting agent because it improves the porosity of the substrate, in addition to being a good carbon source (due to its high content of carbohydrates) (Pandey et al., 2000).

Biological tests are essential to assess the success of the bioremediation process since they can estimate the possibility of the compounds present in the samples to interact with the genetic material of living organisms (Mazzeo et al., 2014), unlike the chemical analyses, which only measure the concentrations of the chemicals present in the environment. In this way, bioassays are essential for the bioavailability assessment of substances present in environmental samples (Moreira et al., 2008) and also for enabling the identification of additive, synergistic, and antagonistic effects of chemical compounds present in complex environmental samples (Pandard et al., 2006).

Plants are excellent genotoxicity bioindicators of soils, as this substrate is the growth medium for the great majority of them (White and Claxton, 2004). Among plants, *Allium cepa* has been considered an efficient test-organism for studies on the basic mechanisms of action of environmental contaminants (Fiskejö, 1985; Bushra Ateeq et al., 2002; Fernandes et al., 2009, 2007), as well as evaluation of the bioremediation process efficacy (Maila and Cloete, 2005; Mazzeo et al., 2015, 2010)

Bioremediation has potential to reduce the toxicity and improve the agronomical quality potential of SS, enabling its application as an agricultural soil conditioner. However, because of the specificity of the detoxification treatments for each SS type, it is now necessary to perform thorough evaluations of the steps involved in bioremediation. Thus, all obtained information in different assays developed with SS, regarding the applied techniques and the type of SS that was used, are important and must be disseminated because they can add relevant data to a better choice of technology to be adopted on the detoxification of different types of sludge produced by WWTPs.

Findings from such studies would likely contribute to the minimization of environmental impacts, which is badly needed. One strategy for the reduction of improper disposal of human waste is the identification of alternative that enable secure and sustainable implementation of SS into agricultural soils. Thus, this study aimed to evaluate the mutagenic, genotoxic, and cytotoxic effects of SS on the test-organism *A. cepa*, as well as estimate the possibility of detoxification of this waste by means of the biostimulation with soil and sugarcane bagasse.

2. Materials and methods

2.1. Material and preparation of the samples

The centrifuged anaerobic SS was collected from the WWTP of Jardim das Flores in the city of Rio Claro, São Paulo, Brazil (latitude 22°24'39"S and longitude 47°33'39"W), in 2011. This WWTP is installed in an area of 40,000 m^2 and only receives domestic effluent, generating 113.53 t of anaerobic SS per month.

For the preparation of the samples, clay soil was used, collected in the Experimental Garden of the University of São Paulo State - UNESP (Campus Rio Claro, São Paulo, Brazil). This soil was previously characterized by Mazzeo et al. (2015) and Christofoletti et al. (2013), regarding the presence of organic matter (ca. 20 g/Kg), electric conductivity (ca. 145 μS/cm), low concentrations of heavy metals, and absence of toxicity, being considered as a reference soil and, thus, adequate for this study. As a decompacting agent, dry and coarsely crushed sugarcane bagasse was used.

In a previous study, Mazzeo et al. (2015) evaluated the bioremediation process of SS mixed with soil in a ratio of 1: 1. Thus, in order to optimize the use of SS, an increase in the proportion of this residue in the tested mixtures was proposed. Thus, the mixtures were prepared in the following proportions: SS pure (SSP); SS mixed with sugarcane bagasse (SS + B; 3:1 [v/v]); SS mixed with soil (SS + S; 3:1 [v/v]); and SS mixed with sugarcane bagasse and soil (SS + B + S; 3:1:1 [v/v/v]).

2.2. Organization of the test

The mixtures were prepared in stainless steel vats (considered as an inert material), with dimensions of 24 cm wide, 20 cm high, and 30 cm long. These vats were prepared to be a suitable environment to estimate the effects of soil bioremediation as well as the potential for the toxic material of the mixture to percolate water during the process of bioremediation. Thus, the vats were assembled with a lower layer (bottom layer) of 2.7 kg of glass beads, to represent the porous layer of the soil and, only subsequently, the mixture under analysis was added. For extraction of the percolate water liquid samples, a hole was made in the bottom of the vats, to which a silicone hose was coupled. During the entire period of bioremediation, the vats were maintained in a covered place and under ambient temperature in the Experimental garden of São Paulo State University, Rio Claro, SP. Weekly, about 200 mL of water was sprayed in each of the vats, to maintain the humidity of the material in order to encourage the growth and maintenance of microorganisms. For each mixture studied, two vats were assembled (duplicate experiments). The periods chosen for evaluation of bioremediation were of 1 (T1), 3 (T2), and 6 months (T3) after the beginning of the experiment.

2.3. Acquisition of aqueous extracts

2.3.1. Aqueous eluate

The acquisition of the aqueous eluate was based on a Brazilian standard (ABNT NBR 10.006, 2004), adding (separately) 62.5 g of each sample (regarding its dry weight) in 250 mL of ultrapure water, followed by constant agitation for 5 min. Unlike the other samples, to obtain the SSP and SS + B aqueous extracts, we added 500 mL and 400 mL of ultrapure water, respectively. After 7 days of decantation at 22 °C, the liquid phase of each sample was collected and filtered through a 0.45-μm membrane.

The dry weight of each sample was obtained by weighing approximately 10 g of each sample in individual treated containers. The entire experiment was performed in triplicate. The drying was carried out at 105 °C for 24 h. Subsequently, a new weighing was performed, and the average of each triplicate was considered as equivalent to the dry weight of each sample.

2.3.2. The percolate water

The leachate was obtained by the collection of the liquid that flowed from each of the vats, due to the natural loss of water from the SS. The collection was carried out as described previously, through a hole in the bottom of the vats.