Chemosphere 148 (2016) 481–486

Contents lists available at ScienceDirect

Chemosphere

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

Cytotoxic, genotoxic and mutagenic effects of sewage sludge on *Allium cepa*

Maria Nilza Corrêa Martins [a], Victor Ventura de Souza [b], Tatiana da Silva Souza [a, b, *]

[a] Programa de Pós-Graduação em Genética e Melhoramento, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, CEP 29500-000, Alegre, ES, Brazil

[b] Departamento de Biologia, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, CEP 29500-000, Alegre, ES, Brazil

HIGHLIGHTS

- Sewage sludge is applied in large scale and continuously in agricultural areas.
- Sewage sludge had a genotoxic effect on *Allium cepa*.
- Sewage sludge genotoxicity should be monitored when it has as destination agricultural soils.

ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form
17 November 2015
Accepted 18 January 2016
Available online 4 February 2016

Handling Editor: Frederic Leusch

Keywords:
Biosolid
Sewage sludge
Chromosomal aberrations
Micronucleus

ABSTRACT

The objective of this study was to ascertain the cytotoxic, genotoxic and mutagenic potential of sewage sludge using *Allium cepa* bioassay. Solubilized and crude sludge from two sewage treatment stations (STSs), herein named JM and M, were tested. In addition, sanitized, crude and solubilized sludge were also analyzed from STS M. The treatments showed positive response to phytotoxicity, cytotoxicity, genotoxicity and/or mutagenicity. Despite negative results for MN F1 (micronuclei counted in F1 root cells, derived from meristematic cells), the monitoring of genotoxic and mutagenic activities of sewage sludge are recommended because in agricultural areas this residue is applied in large scale and continuously. Based on our results we advise caution in the use of sewage sludge in agricultural soils.

1. Introduction

The safe disposal of sewage sludge, a residue from sewage treatment, is of great environmental concern (Singh and Agrawal, 2008). Disposal alternatives of sewage sludge include incineration, use in construction, landfilling, recover degraded areas and application in agricultural soils (Singh and Agrawal, 2008; Fytili and Zabaniotou, 2008).

Disposal of sewage sludge as an organic fertilizer on agricultural fields is due it contains high amounts of macro and micronutrients and organic matter. However, there are risks associated with this practice, which result from the great concentrations of heavy metals, persistent organic compounds and pathogens (viable helminth eggs, thermotolerant coliforms, *Salmonella* and enteric viruses) in sewage sludge. Under the Brazilian legislation, the sludge can be used in agriculture after pathogen decontamination, which is usually accomplished with the addition of calcium oxide (CaO). After treatment, sewage sludge is called sanitized sludge or biosolid (Costa and Costa, 2011).

Application of sludge in agriculture is a pathway for direct contact of crops to toxic chemicals. Thus, genetic toxicity bioassays with higher plants are particularly suitable to evaluate sewage sludge (Hopke et al., 1982; Rank and Nielsen, 1998; Srivastava et al., 2005; Mielli et al., 2009). Since the Espírito Santo State presents agricultural areas with potential application of sewage sludge (Costa and Costa, 2011), this study aimed to analyze the genetic toxicity of this residue from two sewage treatment stations (STSs), through bioassays with *Allium cepa*.

* Corresponding author. Departamento de Biologia, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, UFES, Rua Alto Universitário, s/número Guararema, 29500-000, Alegre, ES, Brazil.
E-mail address: tatianas.souza@hotmail.com (T. Silva Souza).

http://dx.doi.org/10.1016/j.chemosphere.2016.01.071
0045-6535/

2. Materials and methods

2.1. Sewage sludge

Sewage sludge from STSs JM and M were collected according to Associação Brasileira de Normas Técnicas – ABNT NBR 10007 (ABNT, 2004b) in September 2013 and February 2014, respectively. The two STSs have distinct characteristics. The STS JM is located in a municipality with 11,707 inhabitants. There, agriculture (particularly cultivation of conilon coffee) and dairy are the principal economic activities (IBGE, 2014). The STS serves about 60% of the local population (personal communication) and uses the Upflow Anaerobic Sludge Blanket (UASB), in which the treatment occurs in anaerobic environment in a closed reactor. The sewage sludge generated is placed into a tank to dry, and after that it is grounded locally, in the station (personal communication). The region where this STS is located has potential areas where the sludge can be used in agriculture (Taques, 2011). The STS M, on the other hand, is located in a municipality with more inhabitants, 352,104. The main activities there are port-related, commerce, industry, services and business tourism (IBGE, 2014). The STS M is able to process 570 L s^{-1} domestic sewage, and operates through activated sludge in extended aeration. Currently, the sludge it generates is used in agricultural soil, after being sanitized with calcium oxide (CaO).

2.2. Commercial substrate

Commercial substrate Plant Fértil®, commonly used as organic fertilizer of plants, was used as the control.

2.3. Chemical and microbiological analyses

Sludge of both STSs were examined for the following parameters: pH, moisture, total organic carbon, amount of organic matter, concentration of As, Ba, Cd, Ca, Cu, Cr, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se and Zn, thermotolerant coliforms and total coliforms. Analyses were carried out by Agrolab- Análises e Controle de Qualidade LTDA, located in Vila Velha – ES.

The pH, P, K, Na, Ca and Mg of the commercial substrate were measured by Laboratório de Solos from Centro de Ciências Agrárias – Universidade Federal do Espírito Santo. The concentrations of As, Ba, Cd, Cu, Cr, Hg, Mn, Mo, Ni, Pb, S, Se and Zn were determined by Agrolab.

2.4. Solubilization of the sewage sludge and the commercial substrate

Solubilized sewage sludge was obtained following norm ABNT NBR 10006 (2004a), in which 250 g of sewage sludge (dry base) were added to 1000 ml distilled water, agitated mechanically for 5 min and then left to rest for seven days. After decanting the solids, the supernatant was removed to obtain the solubilized sludge. Similar procedure was adopted to obtain the solubilized sludge commercial substrate.

2.5. Treatments

The following treatments were used: distilled water (negative control), trifluralin (190 μL trifluralin/100 mL distilled water) (positive control), solubilized commercial substrate, crude commercial substrate, solubilized sewage sludge, and crude sludge sewage.

Additionally, biosolid samples were prepared from the sewage sludge obtained from STS M. Approximately 200 l sewage were dried over a plastic sheet, to which 30% of calcium oxide (with respect to the dry weight of the sewage) were added for 50 days. In this process, the sludge disinfectants factors are the increase of temperature, the pH change and action of ammonia which is formed from nitrogen (Lima et al., 2011). In this manner, for STS M, besides the treatments cited above, the following samples were also analyzed: solubilized biosolid and crude biosolid.

2.6. A. cepa bioassay

Baia periform onion seeds (2n = 16 chromosomes) from Feltrin® were used. A total of 100 seeds of *A. cepa* were placed on Petri dishes containing the different treatments. Germination took place in a BOD camera at 24 °C. Onion roots *ca.* 1.5 cm in length were collected fixed in a mixture of ethanol and acetic acid (3:1- v/v). Thereafter, roots were washed five times in distilled water during 5 min, hydrolyzed in HCl 1 N at ambient temperature for 20 min and then washed again. Roots tips were cut onto a slide from extraction of their meristematic regions, stained with acetic orcein 2%, covered with cover slips and macerated. To analyze micronuclei in F1 cells (non-meristematic region), the protocol established by Ma et al., 1995 was used.

The slides were analyzed under a light microscope at 400x magnification. A total of 5000 cells were counted per treatment (500 cells in 10 slides). The mitotic index (MI) was used to evaluate the cytotoxic potential of the treatments. The genotoxic potential was assessed by counting mitotic and chromosomal abnormalities (multipolar anaphases, C-metaphase, polyploidy, adhesions, losses and chromosomal bridges) in the cells of the root meristem. Meristematic micronuclei and chromosomal breaks (MN + CB) were measured to assess the mutagenic activity of the treatments. Micronuclei of the F1 region (F1 MN) also considered as result of mutagenic event.

The results were expressed as mean ± standard deviation. Statistical analysis was performed using the Kruskal–Wallis nonparametric test ($p < 0.05$). Bioestat 5.3 software was used.

3. Results

3.1. Chemical and microbiological analysis

Table 1 shows chemical and microbiological properties of commercial substrate and JM and M sludge. The results were compared with the amounts established by the in Brazilian law (CONAMA no 375, 2006).

Heavy metals values of JM and M sludge are below the maximum permitted by law. Sludge JM had thermotolerant coliforms $>5 \times 10^3$ NMP/g of ST, at a value above the maximum permitted by the legislation. On the other hand, sludge M was within the parameters established by CONAMA no 375 (2006).

3.2. A. cepa bioassay

Analysis of the JM sludge showed that the crude sample inhibited the germination of *A. cepa* seeds (Table 2). Solubilized sewage sludge showed a significant increase in mitotic index compared with the commercial substrate and positive control. Also, the solubilized sewage sludge was genotoxic with respect to the crude and solubilized commercial substrates (Table 2). Genotoxicity of the sample was characterized by a significant induction of C-metaphases, polyploid cells and chromosomal bridges (Table 3). The solubilized sewage sludge JM was not positive for mutagenicity (Table 2).

Analysis of the M sludge revealed that the *A. cepa* seeds exposed to crude biosolid failed to germinate (Table 2). Solubilized sewage sludge and solubilized biosolid showed a significant reduction in