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Detoxification of sewage sludge by natural attenuation and implications for its use as a fertilizer on agricultural soils

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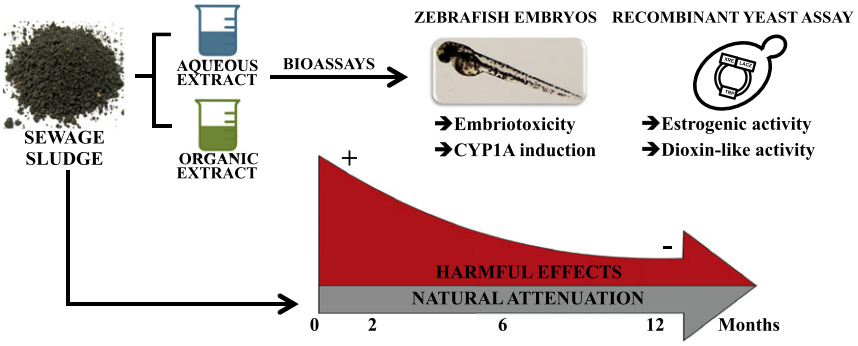
HIGHLIGHTS

- Efficiency of natural attenuation of sewage sludge performed by biological assays.
- Initial samples showed high dioxin-like activity and embryotoxicity in zebrafish.
- SS soil disposal can impact the groundwater by the presence of hydrophilic compounds.
- All the studied effects of SS significantly decreased after six months of attenuation.
- Sewage Sludge requires further decontamination before disposal into the environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Sewage Sludges (SS) from wastewater treatment systems constitute a potential alternative to agricultural fertilizers. However, their use is limited by the presence of toxic substances that may represent significant hazards for the environment and for human health. To test the potential of natural processes to attenuate their putative toxic activities, actual SS samples from domestic sewage were buried in holes in a pollution-free environment for different periods of time, up to one year. Aqueous and organic extracts were obtained after each period of natural attenuation, and their respective toxicity was tested for estrogenic and dioxin-like activity by yeast-based bioassays (ER-RYA and AhR-RYA, respectively) and for general toxicity and teratogenicity in zebrafish embryos. Dioxin-like activity was also tested in zebrafish embryos by monitoring the induction of the marker gene *cyp1a*. Whereas the results showed essentially no estrogenic activity, both dioxin-like activity and embryotoxicity were observed in the initial samples, decreasing significantly after six months of attenuation. Chemical analysis of toxic SS samples showed the presence of low levels of dioxins and furans, and relatively high levels of *m*- and *p*-cresol, at concentrations that only partially justify the observed biological effects. Our data indicates the presence of largely uncharacterized hydrophilic compounds with high biological activity in SS, constituting a potential risk of groundwater pollution upon their disposal into the environment. It also shows that this potential impact may be significantly mitigated by attenuation protocols, as the one presented here.

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1. Introduction

The management of urban residues is a major environmental concern worldwide whose magnitude only tends to increase year after year (Zotos et al., 2009). Sewage sludge (SS) is one of the most worrying components of total waste, as it is generated in large quantities during wastewater treatment processes (Pathak et al., 2009). On the other hand, SS contains high concentrations of organic matter that can be used as a source of nutrients in agricultural soils, an application that has been considered one of the best alternatives for its disposal (Rathod et al., 2009; Zuloaga et al., 2013). However, the accumulation in SS of a wide variety of undesirable organic and inorganic contaminants, as well as of pathogenic microorganisms, represents a limitation for their valorization as an agricultural fertilizer (Urase and Kikuta, 2005; Gibson et al., 2005; Harrison et al., 2006).

Many countries have been encouraging the reuse of sewage sludge in agricultural lands. Nevertheless, to ensure its stability and to prevent harmful effects on the environment, limit values for concentrations of potentially toxic inorganic and organic substances (e.g. heavy metals, policyclic aromatic hydrocarbons, halogenated organic compounds, linear alkyl benzene sulphonates) as well as pathogens in sludge were established. Thus, the disposal of SS in soil must be performed in accordance with the legislation prescribed in each country, for example Directive 86/278/EEC in Europe, Regulation 40 CFR Part 503 in USA and Resolution 375 of CONAMA in Brazil.

Among environmental contaminants, emerging pollutants and endocrine disruptors are attracting much attention due to their high biological activity, usually linked to their specific interaction with vital targets in the living cells. For example, endocrine disruptors, substances able to interfere in the functioning of the endocrine system are of special concern when related to environmental contamination by SS (Giudice and Young, 2011). Among them, natural and synthetic compounds able to interact with the estrogen receptor (ER) at low concentrations may compromise fertility and reproduction of exposed animals, representing a potential risk for human populations (Fatta-Kassinos et al., 2011). Another important class of substances that can be found in SS is dioxin-like compounds, which are capable of binding to and activate the Aryl hydrocarbon Receptor (AhR) (Engwall and Hjelm, 2000). Activation of AhR constitutes the initial step of the metabolic chain leading to toxic effects of a variety of harmful pollutants, such as 2,3,7,8-tetrachlorodibenzo-(*p*)-dioxin (TCDD), co-planar PCBs and benzopyrenes. These effects include immune dysfunction, endocrine disruption, reproductive toxicity, developmental defects, and cancer in vertebrates (Nebert et al., 1993). The interaction of pollutants with both kinds of receptors can be monitored by a variety of single cell assays (Noguerol et al., 2006a, 2006b). In this work we used two yeast-based bioassays, in which yeast strains were genetically modified to reproduce the vertebrate signaling response to either ER- or AhR-ligands. These yeast-based assays, also known as RYA (Recombinant Yeast Assay), have become common tools to detect ER- and AhR-binding activities in a variety of samples and matrices (Noguerol et al., 2006a, 2006b).

Besides being a direct source of soil contamination, SS disposal also contributes to the spread of pollutants in the aquatic environment, mainly by runoff or leaching, potentially affecting aquatic organisms (Eriksen et al., 2009). Zebrafish embryo assays have been widely used for the evaluation of toxicity of environmental samples related to aquatic contamination, due to the high sensitivity of these organisms to xenobiotics (Hallare et al., 2005). In addition, zebrafish embryos can be used to monitor specific toxic responses by the analysis of the induction of pollutant- or stress-related genes. A particularly useful marker is the cytochrome P450 1A gene, CYP1A, whose expression increases in zebrafish embryos (and adults) upon exposure to AhR ligands (Goksoyr and Forlin, 1992; Mcclain et al., 2003; Voelker et al., 2007; Olivares et al., 2013).

A sustainable use of SS will require further decontamination steps before its disposal into the environment (Domene et al., 2008; Ramírez et al., 2008; Rathod et al., 2009; Tas, 2010; Roig et al., 2012). Among the processes indicated for bioremediation of environmental pollutants, natural attenuation (including weathering) is considered an effective and inexpensive pre-processing practice, although its efficiency for removing some recalcitrant, highly bioactive pollutants needs to be examined (Bhupathiraju et al., 2002; Mills et al., 2003). In this context, this study aimed to evaluate the potentially toxic biological activities of SS before and after different periods of natural attenuation in order to verify the effectiveness of this process in promoting a decay of the toxic potential of SS. For this, different endpoints (embryotoxicity, estrogenicity, dioxin-like activities and activation of the CYP1A) were used.

2. Material and methods

2.1. Sludge samples

Samples of anaerobic SS dewatered by centrifugation were collected from the Jardim das Flores wastewater treatment plant (WWTP), located in Rio Claro, São Paulo, Brazil. SS samples were taken as composite samples from different depth of a sludge pile (constituted by 1–2 days production) in two independent sampling campaigns, with an interval of 15 days each.

After sampling, an amount of 8 kg of SS were placed in perforated plastic bags (holes of 0.5 mm diameter and spacing of 1 cm between them) and buried in holes prepared in an environment free of contamination, following the protocol described by Mazzeo et al. (2015). The samples remained buried for periods of 2, 6 and 12 months, in order to allow possible detoxification of the SS by natural attenuation. The periods chosen to perform the attenuation process was based on a previous pilot study, where it was observed that about 2 months of natural attenuation was the minimum period to note any alteration in the toxicity of the samples. The period of 12 months was chosen because it was considered the maximum period for the application of this process becomes feasible to be applied by the WWTP due to the high sludge produced daily amount.

The experiment was conducted in duplicate, analyzing 2 bags for each period of time.

The chemical analyses of the aqueous SS extracts were performed at the laboratory of the Global Analysis and Consultancy (São Carlos—Brazil) following the method proposed by Opeolu et al. (2010), using high performance liquid chromatography with diode array detection (HPLC-DAD). The chemical analyses of the organic SS extracts were carried out at the laboratory of the Analytical Technology Company (São Paulo—Brazil). The analyses were conducted according to the procedure described by USEPA SW-846 (1999). High concentration of *m*- and *p*-cresol and low concentrations of dioxins and furans in these extracts have been reported for these samples (Mazzeo et al. (2015); see Supplementary Table S1).

2.2. Processing and preparation of SS extracts

After each period of natural attenuation, the SS samples were freeze-dried, sieved in an 80 mm sieve and kept in glass flasks, in darkness, at −20 °C, until use.

The protocol NBR10.006 (ABNT, 2004), which is indicated to evaluate the presence of hydrosoluble substances, was used to prepare the aqueous extracts. In individual recipients, 1000 mL of ultrapure water were mixed with 125 g of each freeze-dried sample. After 7 days, the supernatant was collected and filtered in a 0.45 μm membrane.

Organic extracts from SS samples that had undergone 0, 2, 6 and 12 months of natural attenuation were obtained by the Soxhlet method, following the protocol 3540C USEPA (2008). Aliquots of 10 g of each freeze-dried sample were placed in individual cellulose thimbles