Ecotoxicology and Environmental Safety 165 (2018) 367–375

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Toxicity evaluation of leached of sugarcane vinasse: Histopathology and immunostaining of cellular stress protein

Maria Paula Mancini Coelho[a], Jorge Evangelista Correia[a], Louise Idalgo Vasques[a], Ana Claudia de Castro Marcato[a], Thays de Andrade Guedes[a], Miguel Alfaro Soto[b], Juliana Broggio Basso[b], Chang Kiang[b], Carmem Silvia Fontanetti[a,*]

[a] *UNESP - São Paulo State University, IB (Instituto de Biociências), Av. 24-A, 1515, CEP: 13506-900 Rio Claro, São Paulo, Brazil*
[b] *São Paulo State University (Unesp), IGCE (Instituto de Geociências e Ciências Exatas), Av. 24-A, 1515, CEP: 13506-900 Rio Claro, São Paulo, Brazil*

A R T I C L E I N F O

Keywords:
Aquatic toxicity
Gill
Liver
Histology
Immunohistochemical

A B S T R A C T

Sugarcane vinasse is a residue generated at a rate fifteen times greater than the ethanol production. Because of its high organic and micronutrient content, this residue is used as a fertilizer on sugarcane crops. However, when used in large quantities, vinasse can saturate the soil and contaminate nearby water resources by percolation and leaching. Given the proven toxic potential of *in natura* vinasse, the present study aimed to evaluate the toxic potential of leached sugarcane vinasse using Nile tilapia (*Oreochromis niloticus*) as a test organism. A bioassay was performed after vinasse percolation in laboratory soil columns. The bioassay included one control group containing fresh water and two treatment groups, the first exposed to a 2,5% dilution of leached of vinasse and the second to a 2,5% dilution of *in natura* vinasse. After exposure, histopathological analysis was performed in gills and livers, and the latter were labelled for HSP70 proteins. No significant changes were detected in the gills of the exposed fish. However, in the liver, both *in natura* and leached vinasse induced statistically significant histopathological changes. These changes include hydropic degeneration, cell boundary losses, pyknotic nuclei and cellular disorganization. HSP70 expression significant increase in liver of both treatment groups were observed, being higher for the *in natura* vinasse exposed group. Results suggested that both leached vinasse and *in natura* vinasse were toxic, its still able to provoke histological changes and induce the cytoprotective response in exposed fish liver, evidenced by a immunostaining of cellular stress proteins. Thus, in order to reduce its environmental impact, appropriated effluent disposal is essential.

1. Introduction

In the last decade there has been a steady increase in the biofuels demand as an alternative to fossil fuels worldwide. Stimulated by the 1970s oil crisis, the searching for biofuel was intensified in order to increase energy security, offset greenhouse gas emissions and support rural economies, leading to a rapid expansion in the "green" fuels production. However, several environmental impacts related to biofuel production and agro-residues disposal have been reported in the literature, and biofuel usage continues to be a controversial subject (Van der Voet et al., 2010; Tsao et al., 2012; Lazarevic and Michael, 2016).

Sugarcane ethanol represents the main alternative to fossil fuels. Ethanol production from sugarcane biomass includes a distillation process that results in a high amount of liquid by-product residue, the vinasse or liquid stillage, obtained in a proportion of ten to fifteen times greater than the ethanol (España-Gamboa et al., 2012; Christofoletti et al., 2013). In general, it is characterized by acidic pH, high electrical conductivity, high organic content (as organic acids), high chemical oxygen demand (COD) and high biological oxygen demand (BOD) (Moran-Salazar et al., 2016; Soto et al., 2017).

Great environmental disasters happened due to the contamination of surface water sources by *in natura* vinasse. Therefore, since the 1980s vinasse is often used in fertirrigation of sugarcane fields (Fontanetti and Bueno, 2017). This technique is favored by its low cost and the effluent's properties, such as high water and organic matter content, as well as potassium, calcium, nitrogen and other essential plant nutrients (Christofoletti et al., 2013; Prado et al., 2013). Several studies indicate that fertirrigation improves crop productivity, affecting chemical, physical, hydraulic and biological attributes of soil (Goldemberg et al., 2008; Laime et al., 2011; Da Silva et al., 2014).

However, when applied in large volumes, *in natura* vinasse can cause serious environmental impacts. By modifying the soil physical

* Correspondence to: São Paulo State University (Unesp), Department of Biology, IB, Av. 24-A, 1515, CEP: 13506-900 Rio Claro, São Paulo, Brazil.
E-mail address: fontanet@rc.unesp.br (C.S. Fontanetti).

https://doi.org/10.1016/j.ecoenv.2018.08.099
Received 30 April 2018; Received in revised form 21 August 2018; Accepted 27 August 2018
0147-6513/

properties, vinasse may either increase soil infiltration capacity, contaminating groundwater, or diminish it, promoting increased runoff with possible contamination of surface water. In addition, the recharge mechanism of groundwater and aquifers is controlled mainly by rainfall events. Thus, upon reaching the soil containing vinasse, rainwater can infiltrate or drain superficially, contaminating the water resources (Silva et al., 2007).

Considering the different vinasse behaviors in the soil, Soto et al. (2015) performed a mathematical simulation of the water and ionic transport underground flow, hypothesizing the vinasse infiltration due to fertirrigation in sugarcane crops. Results demonstrated that vinasse solute can reach groundwater level in a short time of percolation (1–3 years) depending on its depth (6–20 m), and that surface water resources are also susceptible to contamination in a short period of time.

Considering this scenario, the importance of ecotoxicological studies is highlighted to predict the environmental and human health risks associated with the use of complex contaminants mixtures in agricultural soils. They also support the creation of conservation programs for affected ecosystems. Its instrumental methods of analysis allow an evaluation of the contaminating agents toxicity, where and how they affect living organisms (Magalhães and Ferrão Filho, 2008).

Among the bioindicators, the Nile tilapia (*Oreochromis niloticus*) is widely used as experimental model for studies on aquatic toxicology. This is due to its high growth rates and good reproduction rates in captivity, resistance to handling practices, adaptation to commercial diets and tolerance to various environmental conditions (Figueiredo-Fernandes et al., 2006). Several studies considered the analysis of target organs such as gills and liver appropriated to investigate histological, cellular and physiological changes in organisms exposed to various contaminants (Abdel-Moneim et al., 2012; Ahmed et al., 2013; Rodrigues et al., 2017). Gills are one of the first organs to come into direct contact with substances present in the aquatic environment and are responsible for vital functions. In addition to being the main site of gas exchange, the gills are also involved in the osmoregulation, acid-base balance and excretion of nitrogenous compounds (Machado, 2015). The liver plays a crucial role in detoxification and biotransformation processes. Due to its function, position and blood supply, it is one of the most affected organs by the water pollutants (Van der Oost et al., 2003) and has been used as a reference for the analysis of tissue damage caused by environmental toxic compounds (Ameur et al., 2012; Liebel et al., 2013; Kumar et al., 2016a, 2016b).

Environmental disturbances can also lead to changes in underlying levels of organisms organization, what motivates the use of a biomarker at molecular level to complement this study. Ubiquitous cellular stress proteins (also referred to as heat shock proteins or HSPs) are recognized as being one of the primary defense mechanisms for oxidants, toxins, metals, free radicals, viruses, and their synthesis is generally increased under these stressful conditions (Biekerns, 2000; Ponomarenko et al., 2013). Under normal conditions, constitutive HSPs act as molecular chaperones in important cellular processes such as protein metabolism, cell cycle regulation and apoptosis (Kiang and Tsokos, 1998; Hightower, 1991; Richter et al., 2010). In this group, the HSP70 family (70 kDa molecular weight) stands out because it is highly conserved and extensively studied, being used to monitor environmental pollution due to its cytoprotective role in response to proteotoxic agents (Mukhopadhyay et al., 2003).

In view of the toxic effects caused by vinasse and considering the possible environmental contamination of water resources caused by percolation/leaching of this waste on the soil, the present study investigated that whether after passage through the soil column, the vinasse maintains its toxicity? And can the soil act as a filter for this so toxic residue?

To answer the questions, the vinasse was percolated in the laboratory after construction of soil column system and the residue generated was tested in water using tilapia as a model. For that, the *Oreochromis niloticus* histopathology of gills and liver was evaluated, as well as the immunostaining of HSP70 cellular stress proteins in the liver, after acute exposure of this effluent *in natura* and with leached of vinasse.

2. Materials and methods

2.1. Test organism

Thirty individuals of the species *O. niloticus* (Perciformes, Cichlidae), popularly known as Nile tilapia, were used as test organisms. With an average size of 10 cm and average weight of 40 g, the specimens were obtained from a fish farm. Before exposure, the animals were acclimated for 15 days in 250 L tanks with adequate filtration and aeration system. Fish were fed ration every day up to 24 h before the beginning of bioassays. During the bioassays animals were not fed to not increase the concentration of ammonia in aquariums.

Prior to the beginning of the experiments, the present study was analyzed and approved by the "Comitê de Ética em Pesquisa no Uso Animal da UNESP"- Rio Claro, São Paulo, Brazil (CEUA - IB - UNESP - CRC), protocol no. 6301 from 29.07.2015.

2.2. Vinasse

In the present study *in natura* sugarcane vinasse provided by a power plant located in state of São Paulo was used. It was transported in thermal gallon and stored in a cold room at 4 °C. The leached of vinasse was obtained after the vinasse go through a percolation system in saturated soil (Rio Claro Formation) in the Basin Studies Laboratory (LEBAC) of the Institute of Geosciences and Exact Sciences of UNESP, Rio Claro Campus described in Correia et al. (2017a) as follows: To obtain the leached from the vinasse, a percolation system was developed in the Laboratory of Basin Studies of the Institute of Geosciences and Exact Sciences. The system consists of two vinasse reservoirs with constant stirring to homogenize the solution and avoid vinasse particulates decantation. The soil was compacted at field density in PVC columns (19 cm high and 6.5 cm in diameter) and saturated previously with deionized water. From the reservoir, the vinasse reached the soil with the aid of a modified permeameter to guarantee the vinasse application to the constant and homogeneous load. The leachad was obtained through collectors installed at the end of the column after the cap, composed of a duct, sieve plate with annular and radial grooves and nylon screen, to prevent the passage of soil particles. The leaching tests were performed in a climatized room (20 °C ± 3 °C) and the samples were kept in a cold chamber (4 °C) until the beginning of the experiments.

2.3. Physico-chemical analysis of vinasse

Physico-chemical analysis were carried out to characterize the samples of *in natura* vinasse and leached of vinasse in a specialized laboratory (TASQA Analytical Services Ltd., Paulínia, São Paulo, Brazil). The following parameters were measured: pH, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), calcium, iron, phosphorus, potassium, sodium, and metals (aluminum, barium, lead, cobalt, copper, chrome, strontium, magnesium, manganese, molybdenum, nickel e vanadium, selenium, silicon and zinc).

2.4. Bioassays

The bioassays were set up in 40 L aquaria. Five randomly chosen acclimated fish were placed in each aquarium. The fish were arranged in three groups: control, which received water from an artesian well; test group, which received 2.5% dilution of leached of vinasse; and comparison group, which received 2.5% dilution of *in natura* vinasse. The bioassays were performed in triplicates and the Nile tilapia were subjected to acute exposure (96 h) under constant aeration and temperature (24 °C ± 2 °C) with a 12 h light/12 h dark photoperiod.