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Sugar cane vinasse in water bodies: Impact assessed by liver histopathology in tilapia

Júlia Fernanda Urbano Marinho, Jorge Evangelista Correia, Ana Claudia de Castro Marcato, Janaína Pedro-Escher, Carmem Silvia Fontanetti *

UNESP, Avenida 24 A, 1515, 13506-900–Rio Claro, SP, P.O. Box 199, Brazil

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ABSTRACT

Aquatic ecosystems are the main receptors of toxic substances from human activities. With the increase in sugar cane production, vinasse – the main residue of ethanol production – is a potential contaminant of water resources, due to its high organic matter content. This study was aimed at evaluating the toxicity of vinasse by examining the liver of the fish *Oreochromis niloticus* exposed to different dilutions of sugar cane vinasse (1%, 2%, 5%, 5% and 10%) in laboratory bioassays. Portions of liver were collected and fixed for histological and histochemical techniques to detect total proteins, polysaccharides and lipids. In the histological analysis, the groups treated with vinasse exhibited significant alterations, such as loss of cytoplasmic integrity, loss of cell limit and tissue disorganization. Protein and lipid profiles were not altered. Higher accumulation of polysaccharides was detected in fish exposed to lower concentrations of vinasse, with a gradual decrease in animals treated with vinasse in higher concentrations. We concluded that vinasse has a dose-dependent toxic and cytotoxic potential in water bodies and that the liver is strongly affected when acutely exposed to this contaminant.

1. Introduction

Growing environmental concerns and the interest in renewable resources have boosted the demand for renewable fuels and increased estimates of sugar-ethanol production. This expansion also implies an increase in agroresidues along with their impact on the environment. Thus, effluent monitoring is essential, as they are produced in large quantities and may be disposed or reused inappropriately.

Among agricultural residues, vinasse has been little studied. This effluent is a by-product of ethanol production from different feedstocks, including sugar cane, (Chistofoletti et al., 2013) and has a widely variable composition and properties (Silva et al., 2007). In general, it is characterized by acidic pH and high organic content (Beltran et al., 2005), with dark brown slurry and an unpleasant odor to humans (España-Gamboa et al., 2011; Waliszewski et al., 1997). Ten to eighteen liters of vinasse are produced per liter of ethanol, depending on the distillery equipment. In addition, its high polluting potential is approximately 100 times higher than that of domestic sewage, mainly due to the low pH, high corrosivity and biochemical oxygen demand (BOD) (Freire and Cortez, 2000; Rossetto, 1987; Silva et al., 2007).

Vinasse has also a high value as fertilizer, due to its high organic matter and micronutrient content, and is often reused in fertirrigation of sugar cane fields. However when used in large quantities, vinasse can saturate the soil and contaminate nearby water bodies (Silva et al., 2007), causing serious damage to aquatic life.

Fish have been widely used as experimental models to evaluate the health of aquatic ecosystems and in toxicological pathology (Law, 2003; Ledy et al., 2003; Simonato et al., 2008). According to Figueiredo-Fernandes et al. (2006), tilapia is considered an excellent model for toxicological studies because of its high growth rates, resistance to handling practices, adaptation to commercial diets, good reproduction rates in captivity, and tolerance to various environmental conditions.

The histopathology of fish as biomarker of aquatic pollution has been reviewed by Hinton et al. (1992). These authors reported that the relationship between damage in fish and environmental pollution may be observed in the histopathology of the liver. This organ may exhibit several morphological alterations in toxic conditions, due to its essential role in the metabolism and excretion of xenobiotic compounds (Rocha and Monteiro, 1999).

The present study aimed to evaluate the toxic potential of sugar cane vinasse in water bodies by histopathology of the liver of Nile

* Correspondence to: Universidade Estadual Paulista "Júlio de Mesquita Filho", Av. 24-A, 1515, P.O. Box 199, Laboratório de Histologia, Jardim Bela Vista, 13506-900-Rio Claro, São Paulo, Brazil. Tel.: +55 019 3526-4139.
E-mail address: fontanet@rc.unesp.br (C.S. Fontanetti).

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tilapias after acute exposure to different dilutions of this effluent in laboratory bioassays.

2. Material and methods

2.1. Materials

The Nile tilapia, *O. niloticus* (Perciformes, Cichlidae), was used as test organism. Individuals with an average size of 10 cm were used to avoid intraspecific differences associated with size and age. Farm-raised animals were acclimated in tanks before exposure.

The present study was analyzed and approved by the Committee of Ethics for the Use of Animals of the Institute of Biosciences, UNESP (São Paulo State University), Rio Claro, São Paulo, Brazil, protocol 2866.

2.2. Methods

2.2.1. Physico-chemical analysis of vinasse

Samples of sugar cane vinasse from two different harvests (2010 and 2011) were collected in the city of Araras, São Paulo, Brazil. The effluents were kept in a cold room (4 °C), in the Department of Biochemistry and Microbiology, Institute of Biosciences, UNESP, Rio Claro, São Paulo, Brazil, until the beginning of experiments.

The physico-chemical analysis of the effluent was carried out by the laboratory TASQA Serviços Analíticos Ltda., Paulínia, São Paulo, Brazil to determine its composition. The following parameters were measured: pH, electrical conductivity, hardness, total non-filterable residue, nitrogen, nitrate, and nitrite, Kjeldahl nitrogen, ammonia, calcium, total sulfur, total phosphate, magnesium, potassium, sodium, sulfate, BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand) and metals and metalloids (arsenic, barium, cadmium, lead, copper, chromium, mercury, molybdenum, nickel, selenium, and zinc).

2.2.2. Bioassays with O. niloticus

The bioassays were set up in 40 L aquaria. Five acclimated fish (males and females) were placed randomly ($n=5$) in each aquarium and, to evaluate acute toxicity, the fish were exposed for 96 h. The static exposure system was employed according to studies with fish and other aquatic organisms (Souza; Fontanetti, 2006; Ventura et al., 2008; Oliveira-Filho et al., 2010; Botelho et al., 2012; Marcato et al., 2014). Also, tests were conducted with constant aeration and monitoring of temperature at 24 °C ± ± 2 °C with a photoperiod of 12 h light and 12 h darkness.

The first bioassay was set up as follows:

- Aquarium 1: Control with clean water,
- Aquarium 2: Vinasse at 1%,
- Aquarium 3: Vinasse at 5%,
- Aquarium 4: Vinasse at 10%.

In the second bioassay, the same conditions ($n=5$) and concentrations were used, in addition to vinasse at 2.5%. These dilutions were chosen based on previous studies, which used similar dilutions of vinasse to analyze its effects on different organisms (Algur and Kadioglu, 1992; Kumar and Gopal, 2001).

2.2.3. Dissection of animals

After 96 h of treatment, fish were removed from aquaria, anesthetized, euthanized by pithing with surgical scissors, and dissected in saline solution. The liver was removed and fixed with aqueous Bouin's solution and formol calcium, depending on the stain used. The material remained in the fixative for at least 2 h.

2.2.4. Histology and histochemistry

Portions of liver were dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%). The material was then embedded in resin (Leica Historesin – Embedding Kit) for 24 h in the refrigerator, transferred to plastic molds with resin and later sectioned with a microtome. The sections were stained with hematoxylin-eosin (Junqueira and Junqueira, 1983), according to histological procedures, or prepared for the following histochemical techniques: bromophenol blue for the detection of proteins (Pearse, 1961), Periodic Acid-Schiff (PAS) for neutral polysaccharides, and PAS simultaneously with Alcian blue, for acid polysaccharides (Junqueira and Junqueira, 1983) and Sudan black B and Nile blue for lipids (Junqueira and Junqueira, 1983).

2.2.5. Interpretation of results

Two slides with eight 6 μm sections of each fish were examined, with $n=5$ for each bioassay. Thus, it was analyzed a total of 60 slides and 480 histological sections for each exposure concentration, and the results for the control and treatment groups of the bioassays were compared.

The description and evaluation of the histopathological alterations was adapted from the standardized protocol by Bernet et al. (1999). Importance factors (w) were determined for each lesion, based on its pathological importance, according to how it affects the function of the organ and fish survival. The alterations were previously classified into three importance factors: (1) Minimal pathological importance, the lesion is easily reversible as exposure to irritant ends, (2) Moderate pathological importance, the lesion is reversible in most cases, when the stressor is neutralized and (3) Marked pathological importance, the lesion is usually irreversible, leading to partial or total loss of the organ function. Histopathological anomalies were also scored (a) on a 0–6 scale, depending on the level and extension of the alteration, as follows: (0) no occurrence, (2) mild occurrence, (4) moderate occurrence and (6) severe occurrence. In addition, intermediary values were also included. The index of alteration was calculated by multiplying the importance factor by the score. The total index of each individual, corresponding to the evaluation of histopathological alterations in the liver of each animal examined, was calculated as: $\mathbf{Index_{ind}} = \sum(\boldsymbol{w} \times \boldsymbol{a})$. From the individual indices, the averages and standard deviations were calculated for each group (control and treatments), which were statistically analyzed using Mann–Whitney test, significance level set at 5% and 1%, with the software Bioestat - version 5.3.

This procedure was conducted to analyze liver lesions among treatments. Thus, this standardized method of evaluation allowed a semi-quantitative analysis of the damage to the organs, including the extension, significance, and pathological importance of alterations.

3. Results

3.1. Physico-chemical analysis of vinasse

The analyses revealed that vinasse from both harvests (2010 and 2011) were characterized by a low pH, and high BOD, COD, and potassium. In addition, in the second bioassay, the concentration of sulfate was 400% higher than that of the first one. On the other hand, hardness was lower in the second sample than in the first one (Table 1).

Regarding metals (Table 1), the first vinasse sample (2010) contained different elements, such as barium, copper, chromium, mercury, molybdenum, nickel, and zinc, while the second one had only copper and chromium. However, it should be pointed out that the quantification limits (QL) of the method used by the laboratory for arsenic, cadmium, lead, mercury, nickel, and selenium in the