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Genotoxicity induced by water and sediment samples from a river under the influence of brewery effluent

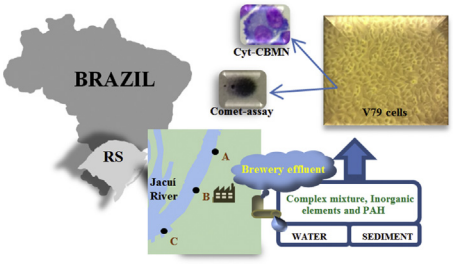
Ana Letícia Hilario Garcia [a, b], Cristina Araujo Matzenbacher [a], Marcela Silva Santos [a], Lismare Prado [a], Jaqueline Nascimento Picada [a], Suziane M. Premoli [c], Dione S. Corrêa [c], Liana Niekraszewicz [d], Johnny Ferraz Dias [d], Ivana Grivicich [e], Juliana da Silva [a, *]

[a] Laboratory of Genetic Toxicology, PPGBioSaúde and PPGGTA, Lutheran University of Brazil (ULBRA), Av. Farroupilha 8001, Prédio 22, Sala 22 (4° andar), 92425-900, Canoas, RS, Brazil
[b] Laboratory of Ecotoxicology, Postgraduate Program in Environmental Quality, University Feevale, ERS-239, 2755, 93525-075, Novo Hamburgo, RS, Brazil
[c] Postgraduate Program in Genetics and Applied Toxicology (PPGGTA) – Chemistry Course, Lutheran University of Brazil (ULBRA), Av. Farroupilha 8001, 92425-900, Canoas, RS, Brazil
[d] Ion Implantation Laboratory, Institute of Physics, Federal University of Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500 – Agronomia, Porto Alegre, RS, Brazil
[e] Laboratory of Cancer Biology, PPGBioSaúde and PPGGTA, Lutheran University of Brazil (ULBRA), Av. Farroupilha 8001, Prédio 22, Sala 22 (4° andar), 92425-900, Canoas, RS, Brazil

HIGHLIGHTS

- Brewery effluent samples induced genotoxicity related to chemical composition.
- Samples obtained near breweries presented increased PAH and metal levels.
- Industrial brewery waste discharges induced DNA damage observed in V79 cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Brewery effluents contain complex mixtures that are discharged into rivers. Therefore, it is necessary to evaluate the genotoxic potential of these effluents. The study evaluated the genotoxicity of surface water and sediment samples from the Jacuí River in the state of Rio Grande do Sul, Brazil, which received effluents discharged from a brewery. The *Salmonella*/microsome test, Comet Assay and Micronucleus test on V79 cells, as well as the element profile (PIXE) and PAHs levels were used for this purpose. The surface water and sediment samples were collected in summer at three sites: 1 km upstream from the brewery discharge site (Site A); in front of the effluent discharge site, after chemical and biological treatment (Site B); about 1 km downstream from the discharge site (Site C). Only a sediment sample from Site A induced a mutagenic effect using the *Salmonella*/microsoma test (TA97a). All three sites presented genotoxicity (A, B and C), both for water and sediments using comet assay, and mutagenicity in the samples from Site B (surface water) and Site A and Site C (sediments) using the micronuclei tests. The results of PIXE and PAHs showed higher levels of elements for samples obtained from sites upstream and downstream from the effluent discharge. Environmental samples consist of complex mixtures of chemicals, and it is

* Corresponding author.
E-mail address: juliana.silva@ulbra.br (J. da Silva).

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difficult to associate DNA damage with a specific element. This study showed that brewery effluent contains metals and PAHs that can induce *in vitro* genotoxicity under the conditions of this study.

1. Introduction

Growing industrialization and urbanization have increased the need for water resources for supply purposes, and their misuse has increased the levels of environmental pollution, both from urban and industrial waste (Islam and Tanaka, 2004; Afroz et al., 2014). In this scenario, the chemical composition of industrial effluents is the main aggravating factor to increase environmental imbalance (Lemos et al., 2008; Rotter et al., 2015).

Brewery effluent from processes used in beer production typically generate high effluent loads, and can alter various parameters in the environment such as chemical oxygen demand, biochemical oxygen demand, suspended solids, oils and greases (Olajire, 2012), the presence of phosphorus, nitrogen (Braeken et al., 2004) polycyclic aromatic hydrocarbons (PAHs) (Moret et al., 1995; Garcia-Falcon and Simal-Gandara, 2005), heavy metals (Huang et al., 2012) and solid waste. Brewery effluents receive chemical and biological treatment before being discharged into the receiving bodies (Yu and Gu, 1996), to meet the limits established by legislation according to the physicochemical standards of regulatory agencies (CONSEMA, 2006a, b; Fillaudeau et al., 2006).

The methods (chemical and physical) usually employed by beverage industries to prepare and maintain their products can induce genotoxic and mutagenic effects (Soares and Soares, 2013; Omoruyi and Pohjanvirta, 2014), which in general are not taken into consideration. Some authors demonstrated the influence of industrial beer effluents inducing mutagenic effects both *in situ* with fish (Odeigah and Osanyipeju, 1995) and in the laboratory with *Allium cepa* (Olorunfemi et al., 2011).

Currently, there are several *in vitro* studies to identifying possible evidence of cytotoxic, genotoxic and mutagenic effects in environmental samples, using bacteria and mammalian cell lines. The bacterial reversion test with *Salmonella typhimurium*, Ames Test or *Salmonella*/microsome assay (Maron and Ames, 1983), is a widely used methodology to evaluate environmental samples and industrial effluents, as it can detect the mutagenicity of different chemical substances and complex biological mixtures which could change the DNA (Vargas et al., 2001; Tagliari et al., 2004; Júnior and Vargas, 2009; Cox et al., 2012). The V79 cell line from Chinese hamster lung fibroblasts is also used to detect DNA damage using both the comet assay and micronucleus test; this cell line possesses a short cell division, is sensitive to chemicals and is easily manipulated (Von der Hude et al., 2000; OECD, 2014).

This study aimed at evaluating the possible genotoxic damage caused by surface water and sediment from industrial brewery effluents collected in the Jacuí River in the state of Rio Grande do Sul (Brazil), using the *Salmonella*/microsome assay (TA97a, TA100 and TA102) and the mammalian V79 cell line; element profile (PIXE) and PAHs levels were also performed using the samples.

2. Materials and methods

2.1. Sampling sites

The Jacuí River occupies 83.5% of the Lake Guaiba basin, Rio Grande do Sul (RS), Brazil, with a 71.600 km^2 area (Floss et al., 2012). Samples of surface water and sediments were collected in March 2015 (summer) at three sites on the Jacuí River (RS/Brazil): (A) Site A, about 1000 m upstream from the brewery effluent discharge point (geographic coordinates 30° 0 ' 8 ″ South Latitude and 51° 12 ' 34" West Longitude), and distant approximately 5 km from the river mouth; (B) Site B, in front of the brewery effluent discharge site, after receiving chemical treatment and biological treatment at the Treatment Plant and from the brewery (geographic coordinates 30° 0 ' 40 ″ South Latitude and 51° 12 ' 48" West Longitude); (C) Site C, 1000 m downstream from the discharge point of effluent into the river, (geographic coordinates 30° 1 ' 20 ″ South Latitude and 51° 13 ' 21" West Longitude). The depths of the collection sites were 4.7 m for Site A, 7.6 m for Site B, and 5.7 for Site C.

Fifteen L of water and approximately 1 kg of sediment were collected from each sampling site in accordance with Standard Methods (2005). Grab samples of surface water (3 samples of 5 L; approximately 20 cm below the surface layer of the water) and sediment (5 samples of 200 g; about 3 cm below the surface of the sediment layer) from each site were obtained at 30 min intervals. The sediment samples were collected using a Van Veen dredge, and of the material retained in the sampler, only the portion that did not come into contact with the sampler walls was collected. After collection, water and sediment samples were mixed prior to distribution into borosilicate containers. One pool of 3 water samples (15 L) and one pool of 5 sediment samples (1 kg) were obtained. The samples were kept at 4 °C and without exposure to light for no more than 24 h to be processed according to the analyses to be performed at the laboratory.

2.2. Sample preparation

2.2.1. Surface water

For cell viability tests, genotoxicity and mutagenicity (micronucleus test and Ames test) surface water samples were sterilized in Milipore filter 0.22 μm, in the laminar flow chamber and separated into bottles and kept frozen at −20 °C until the analyses began (Standard Methods, 2005). Water samples were filtered through qualitative filter paper and irradiated with protons at the Ion Implantation Laboratory of the Physics Institute (Federal University of Rio Grande do Sul - UFRGS) for element profile analysis. Raw water samples were used for physicochemical testing and quantification of polycyclic aromatic hydrocarbons.

2.2.2. Sediment (interstitial water)

The sediment samples were centrifuged at 10.000*g* for 10 min at 4 °C to make it easier to extract interstitial water. Interstitial water samples were sterilized using Milipore 0.22 μm filter, in the laminar flow chamber and separated into bottles and kept at −20 °C (Standard Methods, 2005) for the cell viability, genotoxicity and mutagenicity assays. For element profile analysis, sediment samples were placed in Petri dishes and dried in an oven at 37 °C for 24 h, and then the sediment was sent to the UFRGS Physics Laboratory, where samples were pressed for analysis. Raw sediment samples were used for the identification and quantification of polycyclic aromatic hydrocarbons.