



## Insights about the toxic effects of tannery effluent on *Lithobates catesbeianus* tadpoles

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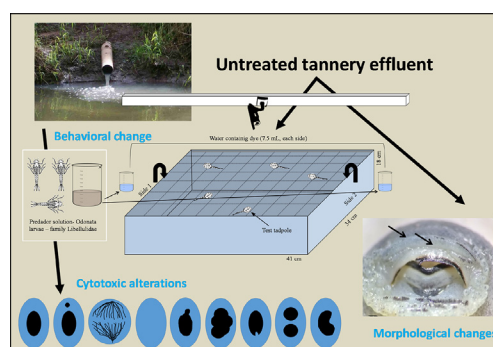
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### HIGHLIGHTS

- Tadpole exposed to untreated tannery effluent (UTE) presented morphologic changes.
- Exposure of *L. catesbeianus* tadpoles to UTE caused cytotoxic effects.
- The total of other nuclear abnormalities was influenced by UTE.
- Tadpoles exposed to the UTE presented abnormal responses in the predator-response test.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Tannery industries are considered highly polluting due to the large production of polluted wastewater [untreated tannery effluent (UTE)]. Although previous studies have already shown the consequences from fish, birds and mammals' exposure to this pollutant, little is known about its toxicological effect on representatives of class amphibian. Thus, we aim at assessing whether short-term UTE exposure, even at low concentrations (0.2% UTE diluted in water), would be able to induce behavioral, morphological and cyto-genotoxic changes in *L. catesbeianus* tadpoles. In order to do so, two experimental groups were set (control and tannery effluent) and exposed, or not, to UTE for seven days. A positive control group (cyclophosphamide) was included in the experimental design in order to assess cyto-genotoxicity. Our behavioral results showed that tadpoles exposed to the contaminant presented abnormal responses in the predator-response test; therefore, it evidenced losses in their capacity to recognize chemical olfactory cues of a potential predator. We also searched for changes in mouth length, in dentition, in body length before and after the eyes of animals exposed to UTE. Besides, we observed higher nuclear abnormality frequency in the circulating erythrocytes of tadpoles exposed to the contaminant, as well as in animals belonging to the positive control group. Some of the observed abnormalities were micronuclei, binucleated, notched, kidney-shaped and blebbed cells, multilobulated nuclei, as well as lower mitotic index. Therefore, our data confirm the hypothesis that UTE causes behavioral, morphological and cyto-genotoxic changes in *L. catesbeianus* tadpoles, fact that opens new perspectives to other investigations about how and which UTE constituents were responsible for the observed effects.

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## 1. Introduction

Tannery industries are an important economic and job creation source in different countries such as India, Brazil, China and Pakistan (Hu et al., 2011; China and Ndaro, 2015; Sabumon, 2016). However, the large amount of residues generated throughout bovine skin processing affects the environmental health, as well as the health of organisms, mainly of those that get in contact with these residues when they are discarded into the environment without any previous treatment (Souza et al., 2016).

Different studies already reported the harming effects of untreated tannery effluent (UTE) exposure by using ecotoxicological models of invertebrate organisms. Oral et al. (2005) showed teratogenicity in *Paracentrotus lividus* and *Sphaerechinus granularis*, besides growth reduction in *Selenastrum capricornutum*. Tigini et al. (2011) evidenced growth reduction in *Pseudokirchneriella subcapitata*, and Júnior et al. (2007) and Tagliari et al. (2004) reported genotoxicity in onion samples (*Allium cepa*), as well as mutagenic activity in *Salmonella/microsome*, respectively. We know that these organisms are good ecotoxicological models; therefore, they allow determining the lethality related to UTE exposure. However, more complex organisms such as those belonging to subphylum Vertebrata (Craniata) (i.e., fish, amphibians, reptiles and mammals), can present different signs, or symptoms, which are not observed in these models when they are in contact with the herein addressed pollutants; therefore, it is necessary conducting deeper investigations in future research.

With regard to vertebrates exposure to UTE, previous studies also confirm its toxicological potential in fish (Aich et al., 2015; Rocha and De Oliveira, 2017; Weldetinsae et al., 2017), birds (Hoffman and Eastin, 1981; Souza et al., 2017a, b) and mammals (Rabelo et al., 2016; Almeida et al., 2016; Silva et al., 2016; Guimarães et al., 2016; Souza et al., 2017a, b; Mendes et al., 2017; Rabelo et al., 2017). Nevertheless, when it comes to amphibians, recent reports were made about some of the effects from UTE on this group (Montalvão et al., 2017a; Montalvão et al., 2017b).

We confirmed the hypothesis that UTE presents clastogenic and aneugenic potential in adult male *Lithobates catesbeianus* in the first study, which was pioneer in showing its cyto-genotoxic effect on these animals (Montalvão et al., 2017a). Animals were exposed to water containing 5% UTE for 30 and 90 days at the occasion of the mentioned study. The second study evidenced that the exposure to the pollutant, even for a short period of time, was able to cause development delay, reduced survival, body mass reduction, body length changes, as well as significant changes in the bilateral features of *Physalaemus cuvieri* tadpoles (Montalvão et al., 2017b). As for the current study, we observed the development of tadpoles since their spawning in water containing 0.2% UTE. Therefore, it becomes evident that the knowledge about the effects these contaminants have on amphibians is quite relevant.

One of the ways to broaden the knowledge of effects caused by UTE on amphibian populations living in aquatic environments receiving these pollutants would be the assessment of some of these animals' "natural" behaviors (inborn/learned), besides the biometric and cyto-genotoxic changes emerging from the exposure to this pollutant. We believe that as the representativeness of biological variables to be investigated increases in organisms exposed to UTE, it is possible having the real dimension of the magnitude of impacts caused by these contaminants on natural ecosystems. Thus, by taking into account that amphibians are good environmental health indicators (Blaustein and Wake, 1990; Wyman, 1990; Lips, 1998), and that the knowledge about the effect of UTE exposure on these animals remains scarce, the aim of the current study was to assess the impact of these residues (diluted in water) on *Lithobates catesbeianus* tadpoles. We assessed whether this contaminant would be able to induce biological changes in the chosen experimental model, even in short periods of exposure at low concentrations of it. We started from the initial hypothesis that the exposure

to UTE may cause behavior, morphological and cyto-genotoxic changes in *L. catesbeianus* tadpoles, because we took into consideration that these residues present quite complex chemical constitution, as well as that previous studies involving other vertebrates have already confirmed the toxic potential of these contaminants.

## 2. Materials and methods

### 2.1. Experimental design

We herein used 45 tadpoles belonging to species *L. catesbeianus*, which, although natural from North America, has been introduced in more than forty countries due to its economic potential (Ficetola et al., 2007). The species is highly tolerant to diseases and infections (Miaud et al., 2016); therefore, it is considered a good experimental model for toxicological studies involving heavy metals and many xenobiotics such as pesticides and contaminants from paper industries and domestic sewage (Wirz et al., 2005; Freitas et al., 2016; Rissoli et al., 2016).

The experiment was performed in laboratory environment under controlled temperature and light conditions ( $24 \pm 2^\circ\text{C}$  and 12 h light/12 h dark). Animals were previously acclimatized to the laboratory conditions for 96 h before the experiment. Next, the tadpoles were distributed into the following experimental groups ( $n = 15$ , in each group):

- Control group – composed of tadpoles housed in boxes containing only dechlorinated water, without any treatment, and
- Tannery effluent group – in which the animals were kept in water containing gross tannery effluent at concentration 0.2%.

Moreover, a positive control group ( $n = 15$ ) composed of tadpoles housed in boxes with water containing cyclophosphamide (at concentration  $40\text{ mg}\cdot\text{L}^{-1}$  - Genuxal®; Baxter Healthcare S/A, São Paulo, Brazil – [drug known for its mutagenic effect, according to Lajmanovich et al. (2014)]) was included in the experiment for cytogenotoxicity analysis purposes.

We inform that the experimental groups were counter-balanced based on the co-variable 'body mas', so that the mean initial biomass of the tadpoles was statistically equal in all experimental groups. Besides, all tadpoles were initially in stage 25G, according to the classification by Gosner (1960).

Tadpoles were exposed to the treatments for seven days, which was a period seen as ecologically relevant in relation to the lifetime of animals belonging to species *L. catesbeianus*. If one considers that at the beginning of the experiment tadpoles were in stage 25G of Gosner's classification (1960), so they presented the frequent search for food in aquatic environment as their main behavioral characteristic, it is possible arguing that the bad environmental condition at this stage could influence their development. On the other hand, it is known that the complete metamorphosis in the studied species (*L. catesbeianus*) can vary from 75 to 90 days, depending on the weather conditions, including light, temperature, food and water quality, among other factors (Cribb et al., 2013). Accordingly, the 7-day exposure may correspond to 7.7–9.34% of the exclusively aquatic lifetime of the species.

The experimental units in the laboratory were set side by side on a workbench. The units were white polyethylene boxes presenting the following dimensions: 18 cm high  $\times$  34 cm wide  $\times$  41 cm long. Each box contained 15 L of dechlorinated water to respect the density 1 tadpole/3 L. Substrates were not placed on the bottom of the boxes; however, compressors were used to constantly oxygenize the water, according to methodology adopted by Costa and Nomura (2016).

The water in the boxes had 50% of its total volume replaced only once throughout the experiment. The replaced volume had the same concentration of the treatments (0.2% UTE in the tannery effluent group and  $40\text{ mg}\cdot\text{L}^{-1}$  of cyclophosphamide in the positive control group). The cleaning of the boxes was performed on a daily basis, in

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