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Evaluation of the toxic potential of coffee wastewater on seeds, roots and meristematic cells of *Lactuca sativa* L.

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ABSTRACT

Coffee wastewater (CWW) is an effluent produced through wet processing of coffee containing high concentration of organic matter, nutrients, salts and also agrochemicals. It is released directly into the argillaceous soil or into decantation tanks for later disposal into soils, by fertigation, subsurface infiltration or superficial draining. However, this practice is not followed by the monitoring the toxicity potential of this effluent. In this sense, the present work aimed to evaluate the phytotoxic, cytogenotoxic and mutagenic potential of CWW on seed germination, root elongation and cell cycle alterations in the plant model *Lactuca sativa* L. The effluent (CWW) collected was diluted in distilled water into six concentrations solutions (1.25%, 1.66%, 2.5%, 5.0%, 10%, 20%). A solution of raw CWW (100%) was also applied. Distilled water was used as negative control), and the DNA alkylating agent, metilmetano sulfonate (4×10^{-4} M) as positive control. Physico-chemical parameters of the CWW was accessed and it was found that the effluent contained total phenols and inorganic matter in amounts within the limits established by the National Environment Council (CONAMA). Nevertheless, the biologicals assays performed demonstrated the phytotoxicity and cytogenotoxicty of CWW. Seed germination was totally inhibited after exposure of raw CWW. In addition, a decrease in seed germination speed as well as in root growth dose-dependently manner was noticed. Moreover, nuclear and chromosomal alterations were observed in the cell cycle, mostly arising from aneugenic action.

1. Introduction

According to Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) (2015), Brazil is considered the largest coffee producer and exporter worldwide, being also the second greatest consumer of this product. Fifteen federal states stand out as largest national coffee producers, with Espírito Santo occupying the second position.

High quality of the coffee grains and of the subsequent drink is a requirement of the consumer market; based on this, the wet processing post-harvest is preferred. This process yields a drink of higher quality, as it maintains the sensorial characteristics of the coffee and reaches good prices on the market (Almeida and Silva, 2006). Moreover, it has the advantage of decreasing the drying yard area and time (Ricci et al., 2004). However, this practice is based on the use of water, which generates high volume of effluent, denominated coffee wastewater (CWW).

Shanmukhappa et al. (1998) affirm that, in India, depending on the adopted method and water availability, the amount of the liquid used for wet coffee processing can vary between 8000 and 20,000 L/ton, and further estimate the production of effluents to be around 2078×10^6 m^3. Performing the physico-chemical characterization of the effluent generated at each stage of the wet processing of coffee grains, Rigueira et al. (2010) verified the production of 20,000 L of CWW.

CWW is characterized by high concentration of organic matter, nutrients, salts and agrochemicals (Matos et al., 2007). It presents high acidity, which may result in fermentation accompanied by odor and dark color, with high biological and chemical oxygen demand (Kulandaivelu and Bhat, 2012). Hence, the inadequate disposal of this effluent into the environment may pose a pollution risk to the soil and/or water (Matos et al., 2007). One of the ways of handling CWW is the disposal into soils, by fertigation, subsurface infiltration or superficial draining (IDAF, 2014); however, there are no works demonstrating its toxic potential for the biota.

According to Zagatto and Bertoletti (2006), the most efficacious strategy to evaluate the environmental risk of an effluent is the

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joint application of physical, chemical and ecotoxicological analyses. The chemical and physical parameters are often insufficient to characterize the potentially toxic effect of the effluent on living organisms. Thus, the combination of physico-chemical analysis with biological assays provides a more real approach regarding the toxic potential and the properties of complex mixtures (Andrade-Vieira, 2012).

This way, the use of bioindicators to evaluate the impact of certain substances on aquatic life is crucial in studies aiming to assess the effect of pollutants on living organisms. Among the models available to investigate the potentially toxic activity of effluents released into the environment, recommended by environmental organism such as the Environmental Protection Agency (US EPA), are the higher plants (Grant, 1999). The available assays identify the genotoxicity and mutagenicity of environmental pollutants, as is the case of CWW, and are able to detect the toxic potential of an effluent even when found at low concentrations in the environment (Leme and Marin-Morales, 2009).

The mechanism and processes of cytotoxicity, genotoxicity and mutagenicity trough bioassays with higher plants are accessed by parameters such as MI, chromosome aberrations and micronucleus, respectively. These evaluations may allow establishing the mechanism of action of the studied substance or compound on the genetic material, which can be defined as aneugenic (related to some cell structure as mitotic fuse or proteins as histones) or clastogenic (related to the induction of DNA and chromosomes breaks) (Leme and Marin-Morales, 2009; Freitas et al., 2016). These mechanisms accessed by cytogenetic tests could be complement to the evaluation of plant development and growth by means of seed germination and root development analyses, determining the phytotoxicity of the contaminant, performed (Andrade et al., 2010).

Moreover, bioassays using higher plants have low cost; present good correlation with other test models and systems, such as animal ones; and rarely provide false results, being very reliable and constituting adequate candidates for programs of genotoxicity monitoring. Moreover, the chromosomes of higher plants indicated as models are large and of easy visualization, which facilitates the cytological analyses (Fiskesjö, 1985).

Seeing that studies on the toxic potential of CWW are lacking, this work aimed to evaluate the toxic potential of CWW derived from wet coffee processing, using the plant species *Lactuca sativa* L. as model. The species has prove to be an efficient system for ecotoxicological studies combining analyses on the chemical composition of effluents with biological assays, including cytogenetic analyses (Palmieri et al., 2014).

2. Material and methods

2.1. Collection of coffee wastewater

Collection of the material was carried out inside a rural property (UTM DATUM WGS84) where wet processing of coffee is performed, located at the north of the city of Muniz Freire, south of the state of Espírito Santo (ES, Brazil), in the period between May and August of 2013. In this period, two collection were carried out on coffee waste water storage tanks. The samples were maintained on plastic disposable flasks under refrigeration until the physic-chemical and biological analysis performed.

2.2. Analyzed physic-chemical parameters

The physico-chemical analysis of the effluent was performed according to the parameters determined in regulation 430/2011 – Effluents of the National Environment Council (CONAMA). The measured parameters were pH, temperature, total dissolved solids, conductivity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total phenols, and dissolved inorganic matter. Five samples (three samples of 1000 mL, one of 300 mL and one of 200 mL) of the collected CWW were analyzed followed INMETRO standards sampling method for wasterwater (IT-COL-002). The assay was audited by quality-control system based on the standard NBR ISO/IEC 17025:2005.

2.3. Dilutions of coffee wastewater

To investigate phytotoxicity, genotoxicity and mutagenicity, the collected effluent (CWW) was diluted in distilled water to provide six concentrations: 1:80 (1 L of CWW and 80 L of distilled water) or 1.25% CWW; 1:60 or 1.66% CWW; 1:40 or 2.5% CWW; 1:20 or 5.0% CWW; 1:10 or 10% CWW; and 1:5 or 20% CWW. Also the effect of raw effluent (100% CWW, undiluted) was evaluated. Distilled water was used as negative control (NC) and the DNA alkylating agent, metilmetano sulfonate (MMS) (4×10^{-4} M) as positive control (PC).

2.4. Experiment assembly

The experiment was developed in completely randomized design (CRD), consisting of nine treatments, each with five repetitions. Each repetition was represented by one Petri dish containing 50 seeds of the test model L. *sativa*, distributed onto germination paper moistened with approximately 5 mL of the test solutions.

2.5. Germination and root growth test

The Petri dishes containing the treatments were kept in BOD chamber at 24 °C for 48 h. The number of germinated seeds was determined at the times 8, 16, 24, 32, 40 and 48 h. Based on these data, the germination rate (GR), corresponding to the percentage of germinated seeds in each treatment, and the germination speed index (GSI) were obtained.

GR was calculated using the formula:

$$GR = TN\ x\ 100/N,$$

where TN represents the total number of germinated seeds and N the total number of seeds in each dish.

GSI was calculated using the following formula:

$$GSI = (N_1{*}1) + (N_2{-}N_1){*}1/2 + (N_3{-}N_2){*}1/3 + \ldots\left[N_x{-}(N_{x-1})\right]{*}1/y,$$

where N_x corresponds to the number of germinated seeds in a given period and *y* indicates the total evaluated time, which amounted to six time points in this study.

After 48 h, the length of all roots emitted by the seeds in all treatments was determined with a digital caliper, so as to obtain the mean root length (MRL).

$$MRL = RL_T/N,$$

where RL_T is the sum of the root lengths of the treatment and N is the total number of germinated seeds.

2.6. Cytogenetic test

After 48 h of exposure to the control and CWW treatments, the roots were collected and fixed in ethanol: acetic acid (3:1) solution for at least 24 h. Next, they were washed and hydrolyzed in 5N HCl at room temperature. The slides were prepared by squashing technique with 2% acetic orcein and sealed with nail polish, being stored in moist chamber. A total of 1000 cells were counted per slide, with two slides per Petri dish, amounting to ten slides and

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