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COMPARATIVE ECOTOXICOLOGICAL EVALUATION OF TANNIN COAGULANTS FROM BLACK WATTLE AND FERROUS ALUMINUM SULFATE IN THE TREATMENT OF SLAUGHTERHOUSE EFFLUENT

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Abstract

Ecotoxicological assessments are crucial for environmental monitoring as they can pre-dict the adverse effects of ecotoxins, such as coagulants, on the ecosystems using contamination bioindicators. This study evaluated the ecotoxicity of two types of coagulants, an inorganic coagulant, ferrous aluminum sulfate (SF), and a biocoagulant based on tannins extracted from black wattle, used in the treatment of cattle slaughterhouse effluents. The planaria *Girardia tigrina*, which is used as a bioindicator of toxicity in freshwater ecosystems, was used as the test organism for this study. To select the appropriate tannin coagulant for the effluent under study, two types of biocoagulants were investigated: Tanfloc SG (SG) and Tanfloc MTH (MTH), with SG coagulant showing better performance (98.5 % turbidity removal). In the ecotoxicological test, SG and SF coagulants were compared. Both the tannin-based coagulant SG and the inorganic SF were effi-cient for the treatment of the evaluated effluent, yet their lethal concentrations (LC50 - 96h) with regard to *G. tigrina* was 32.24 % and 42.24 %, respectively. Thus, our results suggest that the effluent treated with the tannin-based coagulant SG showed greater toxicity to *G. tigrina* than the inorganic coagulant ferrous aluminum sulfate.

Keywords: ecotoxicology, black wattle tannin, ferrous aluminum sulfate, slaughterhouse effluents, bioindicators, biocoagulants.

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Introduction

Agricultural reuse is classified in the form of direct, non-potable water reuse and has a fundamental role in the management of water resources. The possible forms of water reuse to which waste water may be subjected to, however, depends on the physicochemical and microbiological characteristics of the effluents in the waste water (Helmecke, Fries and Shulte, 2020).

Planned reuse is an alternative to increase water suitability for consumption, since the withdrawal of water from the reservoirs reduces and, consequently, becomes more susceptible to pollution. Acceptance and the use of properly treated effluents has increased in recent years due to high water demand. As agricultural consumption is tolerant to the use of properly treated effluents, the use of good quality water would be intended only for human consumption, which requires a higher quality of water. Some industries seek to invest in research to treat their effluents and improve their quality, as reaching the release standards is a difficult task for industries (Fico *et al*., 2022).

Among the possible measures to be adopted, we highlight the use of chemical coagulants, particle breakage by pumping, disintegration by ultrasound, filtration of the primary effluent, and physical-chemical processes. Physicochemical systems, as a preliminary treatment of the effluent, allow greater control due to the adjustment of dosages, although there is a greater generation of sludge (Bustillo-Lecompte and Mehrvar, 2017). For some effluents, such as cattle slaughter, the chemical characteristics of the sludge (high concentration of proteins) allow its reuse in the manufacture of animal feed, adding value and reducing the volume of waste (Limeneh *et al*., 2022).

The processing of products, that are of animal or vegetable origin and are mainly made up of foodstuffs, by agro-industries use fermentative processes that generates liquid residues rich in organic matter, nutrients, and salts. Even after treatment, the water bodies present potential for pollution, mainly due to the presence of nitrogen and phosphorus. Domestic and industrial sewage are characterized as point sources, located and well identified as being responsible for significant depletion of oxygen in water courses, and an increase in solids, pathogenic microorganisms and nutrients (Demirel and Yenigun, 2004, Menegassi *et al*, 2020).

Given this, the objective of this study was to evaluate the optimal dosages for the tannin coagulant of black wattle and ferrous aluminum sulfate by performing ecotoxicological tests using the planaria *Girardia tigrina* as a bioindicator, to elucidate the toxic potential of the treated effluent for each coagulant.

Aluminum sulfate, in particular, has been used for more than 100 years all over the world and in different treatment system concepts, aiming at the removal of particulate matter, colloidal matter and organic substances via chemical coagulation. However, the extensive use of aluminum

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sulfate has been discussed due to the presence of remaining aluminum in the treated water and in the sludge generated at the end of the process, often in very high concentrations, which makes it difficult to dispose of it in the soil due to contamination and the accumulation of this metal. Thus, the use of biocoagulants has become an alternative to replace inorganic coagulants. Black wattle tannin is a natural coagulant extracted from the tree species *Acacia mearnsii* and can act in colloidal systems, neutralizing charges and forming bridges between these particles, this process being responsible for the formation of flakes and consequent sedimentation. Among its properties, tannin does not change the pH of the treated water, since it does not consume the alkalinity of the medium, at the same time that it is effective in a wide pH range, from 4.5 to 8.0 (Azreen *et al*., 2021).

Owing to their high regenerative ability, the planarian serve as model organisms to study the mechanisms of morphological and functional restoration (Kreshchenk *et al*., 2008). Furthermore, they are used in aquatic toxicology because they are sensitive to a variety of pharmacological and toxicological agents, and biochemical and physiological analogies can be made with the susceptibility of animals superior to pollutants. They are important components of freshwater ecosystems, have a wide geographical distribution, are sensitive to pollutants, and are easy to create and maintain in laboratories, and were thus chosen for this study. In addition, planarians are also saprophagous, contributing to the balance of decomposer communities existing in the biosphere (Barros *et al*., 2006 and Macedo *et al*., 2019).

Thus, the present study compared the acute toxicity (LC50) of bovine slaughterhouse effluent (CSE) treated with ferrous aluminum sulfate versus CSE treated with a tannin-based coagulant. This study is important because it contains information on the inorganic coagulant ferrous aluminum sulfate and a biocoagulant based on black wattle tannin. The data reported in this study contributes to the understanding of the effects on the environment resulting from the release of effluents treated by these coagulants, adding ecotoxicological data in relation to the organism *G. tigrina*, a relevant bioindicator for freshwater ecosystems, as it is part of the food chain in many trophic levels.

Materials and methods

Effluent collection

The effluent was collected after railing the raw effluent. The collected effluent was characterized using the following physicochemical parameters: turbidity, dissolved oxygen (OD), total dissolved solids (SDT), pH, conductivity, biochemical oxygen demand (BOD), ultraviolet absorbance at 254 nm (Abs 254 nm), and total solids (ST), according to the methodology described in the Standard Methods of the Examination of Water and Wastewater (APHA, 2017).

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Coagulation/flocculation tests followed by flotation

Coagulation/flocculation and flotation tests were performed using jar test equipment. The flotation process was spontaneous and did not involve recirculation of water saturated with dissolved air.

First, the most suitable biocoagulant for the effluent was evaluated by testing Tanfloc SG (100, 200, and 300 mg/L) and Tanfloc TMH (200 and 300 mg/L) (PERLOTE *et al*., 2019). The operating conditions for these tests were as follows: fast mixing gradients of 260 rpm for 10 s and slow mixing of 30–50 rpm for 10 min. Collections were performed at a flotation speed of 0.23 cm/min.

The efficiency of the ferrous aluminum sulfate (SF) coagulant at a concentration of 300 mg/L and SG (200 mg/L) was evaluated for further ecotoxicological testing. An SF dosage of 300 mg/L was used to reproduce the process used by the company supplying the effluent.

The characteristics of the treated effluent for both coagulants were then measured. Notably, the effluent used in the ecotoxicological tests was filtered through cotton because of the large volume required. Filtration on paper membranes is not feasible because of the rapid clogging of the filter medium. The non-filtration of the treated effluent resulted in the adhesion of suspended solids in planarians and death in less than 24 h. As the objective of this work was to evaluate the effluent and not the sludge, filtration was carried out.

For filtration, a portion of 1.5 g of 100% hydrophilic cotton was used in a glass funnel. The volume of 1.5 liters of effluent, after the flotation process, was collected from the bottom of the jar test, avoiding to collect the floated sludge.

Bioassays with Girardia tigrina

Before the ecotoxicological assays, coagulant efficiency tests were carried out so that the dosages used provided physicochemical characteristics similar to the treated effluent.

For the bioassays, the test organism *G. tigrina* (planaria) was used to evaluate the acute effect of the lethal concentration (LC50) of the effluent treated with two different coagulants: ferrous aluminum sulfate and tannin (SG Tanflonc®).

The planar culture of *G. tigrina* was obtained from the Ecotoxicology Laboratory at the University of São Paulo and cultivated at the Ecotoxicology Laboratory (Research Group on Functional and Applied Ecology) at the Federal University of Tocantins (UFT - Campus Gurupi). Individuals of *G. tigrina* were kept in culture boxes, containing 1.5 L of ASTM medium (ASTM, 1980) and provided with constant aeration. This preparation was maintained in an air-conditioned room at 22 ± 1 °C. The animals were fed bovine liver and had their medium renewed on a weekly basis.

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Acute (lethal) test
For the acute test, organisms of 8 mm (± 0.1 mm) in length were selected. The organisms selected for the test were deprived of food the week before the experiments were carried out. To determine the lethal concentration (LC50), different volumes of refrigerated effluent treated with two different coagulants were used: ferrous aluminum sulfate and tannin. The experimental solutions were prepared by diluting the stock solution in ASTM medium. For the control solution, only the ASTM medium was used. Physicochemical parameters such as dissolved oxygen, pH, conductivity, and temperature were measured from these solutions. For each test, 45 Petri dishes were used, with five replicates for each concentration (containing five planarians) in each treatment, containing 20 mL of the experimental solution, totaling 225 organisms for each test (Figure 1). The organisms were exposed for 96 h in a static system (22 ± 1 ℃), in the dark, or without food. Thus, mortality was verified after 24, 48, and 96 h of exposure by counting the number of static/dead organisms in each repetition to determine the LC50. Physicochemical parameters such as dissolved oxygen, pH, conductivity, and temperature were measured from the experimental solutions of each concentration. Planarians that showed immobility when exposed to light or when the body (totally or partially) showed degeneracy, were considered dead.

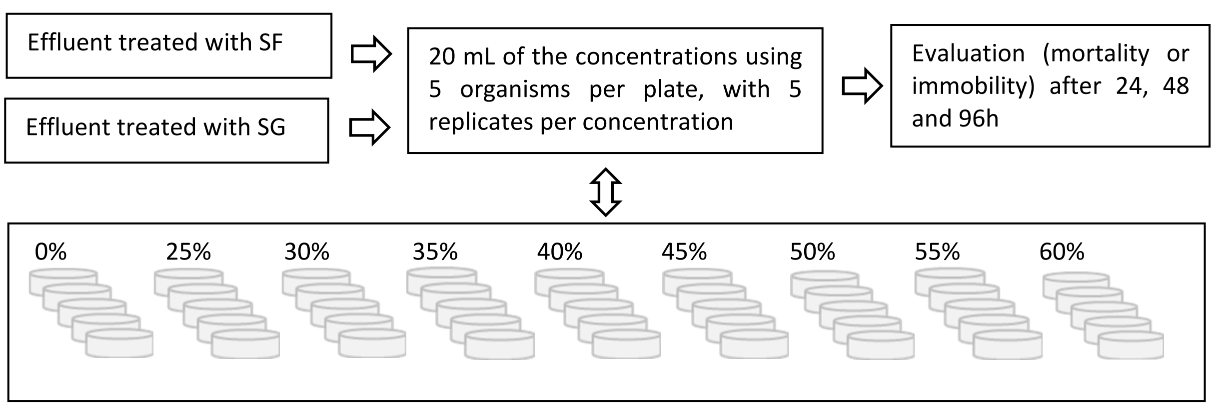

Figure 1. Bioassay scheme.

Statistical analysis
Analysis of the acute tests were performed using the Minitab ® program through probity analysis. The analysis was also performed using GraphPad Prism software version 6.0 for Windows (GraphPad Software, La Jolla, California, USA).

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Results and discussion

Coagulation/flocculation/flotation tests

The physicochemical characteristics of the collected effluents are shown in Table 1, which describes the minimum and maximum values of the samples collected in relation to each evaluated parameter.

The turbidity parameter showed greater variation between collections, therefore, the results of the coagulation/flocculation/flotation tests indicate the initial turbidity values of the sample. The results used to select the biocoagulant are shown in Figure 2.

The results obtained with the MTH coagulant showed lower turbidity removal efficiency than the SG coagulant for the effluent used in this study. In addition, the manufacturer recommends that tannin-based coagulants should not be applied at concentrations greater than 200 mg/L.

Thus, comparative results with the SF coagulant were performed with the SG coagulant at a concentration of 200 mg/L. Figure 3 presents the turbidity removal results comparing SF 300 mg/L and SG 200 mg/L, both evaluated in three different collections.

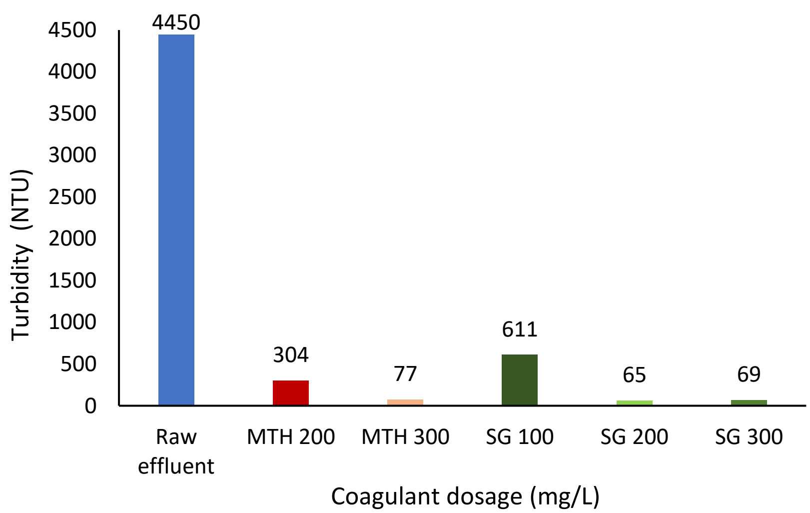

Figure 2. Remaining turbidity using the tannin-based coagulants TMG and SG in diferents dosages.

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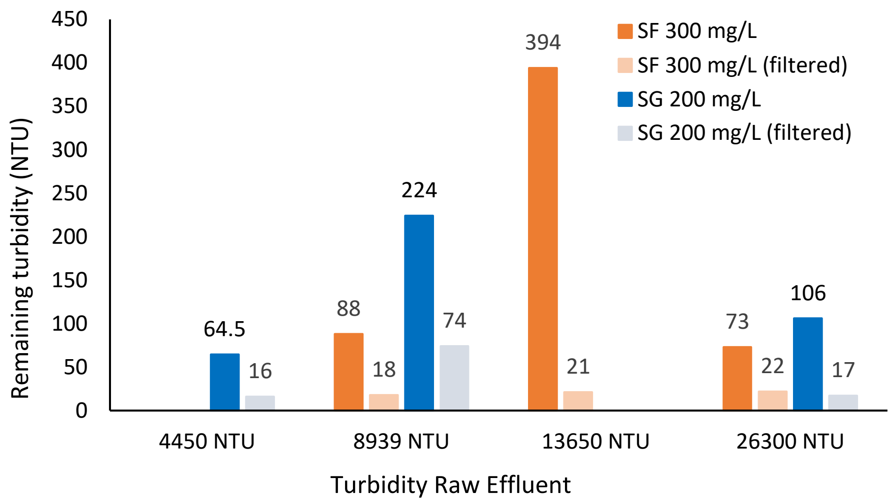

Figure 3. Efficiency of removing turbidity between coagulant SG and SF.

After the coagulation/flocculation/flotation tests, the treated effluents were filtered to remove larger suspended solids, which significantly affected the ecotoxicological test. Thus, the treated effluent (Table 1) was characterized after the filtration process to represent the evaluated effluent in a coherent manner.

Table 1. Physical-chemical characterization of the raw effluent and after the coagulation / flocculation / flotation / filtration process used for ecotoxicological tests.

Parameters	Raw effluent		SF	SG
	Min	Max*		
Turbidity (NTU)	4450	26300	18	74
DO (mg/L)	<1	2.92	-	-
Total Solids (mg/L)	-	-	769	770
pH	5.9	6.5	5.9	6.4
Conductivity (µS/cm^2)	1170	1640	1222	1238
Total dissolved solids (mg/L)	585	820	611	619
BOD (mg/L)	4935	5926	<400	356
Abs (254 nm)	-	1.245	0.803	0.764

**Effluent used for the application of coagulants.*

The pH values presented in Table 1 refer to the samples after the coagulation/flocculation/filtration process. The reduction in pH in the SF test is justified by the fact that SF is an acidic salt, as shown in Equations 1 and 2. The coagulant SG did not result in a significant change in the final pH.

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$$8\ Al_2(SO_4)_3 + 6H_2O \leftrightarrow 2\ Al(OH)_3 + 6H^+ + 3SO_4^{-2}$$ **Equation 1**

$$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH) + 3H^+$$ **Equation 2**

The dosages used promoted similar removal efficiencies for the coagulants, for example, the turbidity removal was greater than 99% for both treatments, as well as the BOD removal was greater than 93% for SF and SG. Dissolved solids removal and electrical conductivity were also close to 25% for both.

Turbidity removal efficiencies greater than 90% were observed in bovine slaughter effluents treated with SG at dosages of 200 to 300 mg/L by Oliveira *et al*. (2018) and with dosages from 50 mg/L by Parlote *et al*. (2019) in slaughter effluent collected after biological treatment. In the work by Perlote *et al*. (2019) the authors indicated the dosage of 250 mg/L so that the treated effluent obtained color removal above 90%.

Notably, although the use of the biocoagulant represents the addition of an organic load in the effluent, an increase in the BOD and Abs 254 nm was not observed. The other parameters were similar and showed similar efficiencies. The UV-Vis spectra of the effluent and treated effluent are shown in Figure 4.

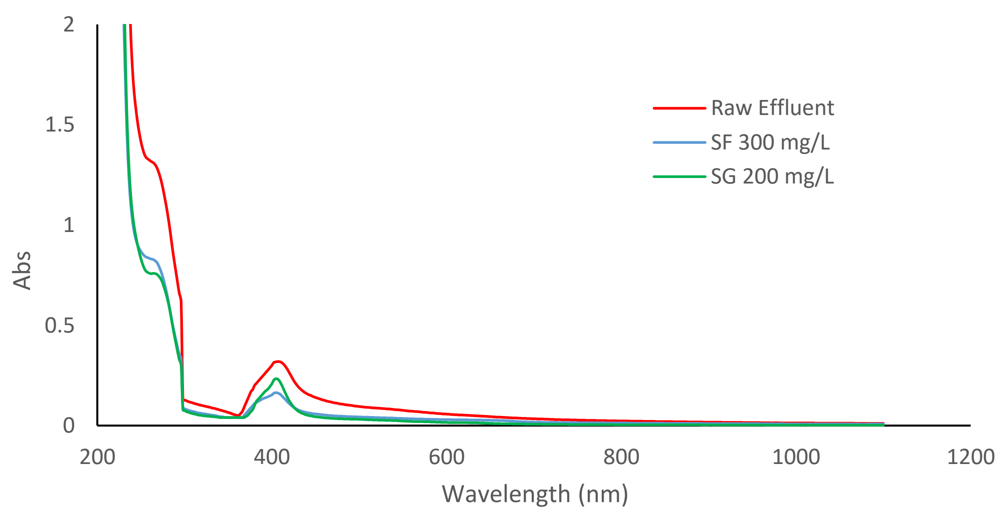

Figure 4. UV-Vis spectrum of the effluent before and after treatments.

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The most common chromophores in oils and fats from bovine tallow are chlorophyll, carotenoids, flavins, tocophenols, phosphatides, and sterols, with carotenoids being mainly responsible for the yellow-red color in animal fat (Patterson, 1992). Bovine tallow is mostly composed of fatty acids: palmitic acid (23–29 %), oleic acid (20–35 %), and stearic acid (26–45 %).

According to Pantoja (2013), the characteristic bands of oleic and palmitic acid are close to 350 and 375 nm, respectively, and those of β-carotene are between 400 and 540 nm, which can explain the bands in Figure 4. Figure 5 shows images of the effluents after flotation.

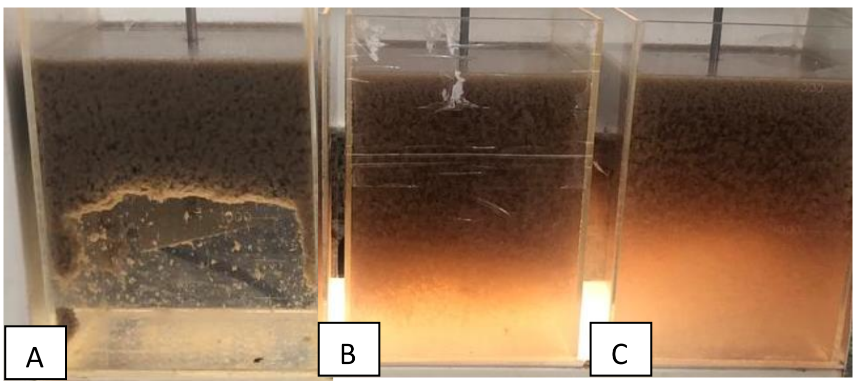

Figure 5. Test with coagulants from SF (A) and tanine SG (B) and MTH (C).

In the SF test, it is possible to observe coagulation by means of a scanning mechanism in which precipitates ($Al(OH)_3$) form, in the case of the SG test, the coagulation mechanism can be represented by the formation of bridges, characterized by involving the use of polymers with large molecular chains as a coagulant. In Figures 5B and 5C, coagulation by charge neutralization can also be seen, which may not form flocs suitable enough to settle or float, with direct filtration being recommended as a subsequent process, so that destabilized particles can be retained in the granular medium of filters.

Ecotoxicological tests

Acute test with planaria

The results are shown in Figure 6. According to the acute ecotoxicological test, a solution containing 42.24 % of the effluent treated with ferrous aluminum sulfate diluted in ASTM medium was necessary for 50% of the test organisms to die after 96 h of exposure. For the coagulant based on tannin SG, 32.42 % of the treated effluent was necessary. Thus, it was possible to verify that the effluent treated with the tannin-based coagulant SG showed greater toxicity than the inorganic coagulant ferrous aluminum sulfate in relation to the bioindicator *G. tigrina*.

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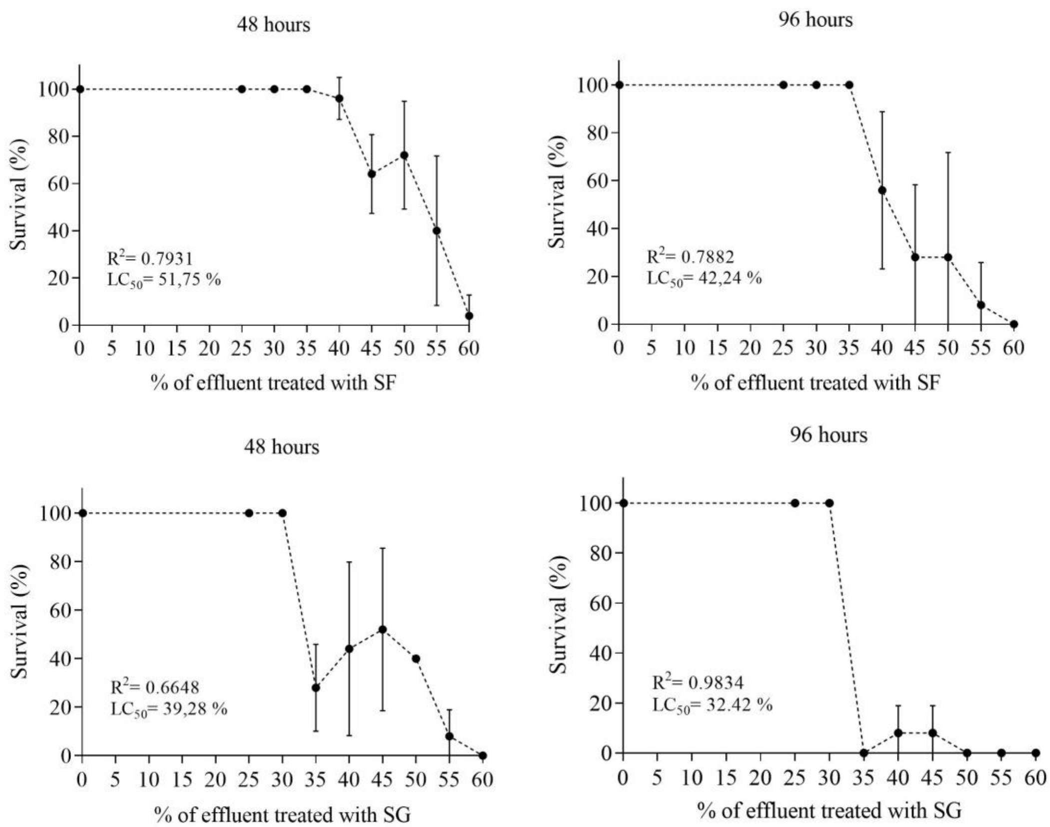

Figure 6. Acute test with planarians (±sd).

The ability of plants to protect against pests is attributed to tannins. According to (Berbehenn and Constabel, 2011), tannins are especially prone to oxidation in insects, forming semiquinone and quinone radicals as well as other reactive oxygen species, and lead to tannin toxicity, which is believed to result from the production of high levels of reactive oxygen species. In microorganisms, inhibition by tannin compounds is due to hydrogen bonding with vital proteins such as enzymes. Tannin polymerization can result in the toxification of tannin monomers and detoxification of tannin oligomers, and this polymerization can occur through contact between tannin and air (Field and Lettinga, 1992).

The final quality of the effluent after the treatments were similar in most of the evaluated parameters (Table 1). However, this was not observed for the turbidity parameter, as the effluent treated with SG showed turbidity 4 times greater than the effluent treated with SF. The effect of particles (turbidity) could be observed in preliminary tests with the effluent without filtering.

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Without filtration, the flakes adhered to the planaria and the mortality rate was very high, which is why filtration was chosen after the coagulation process. In planarians, oxygen is absorbed across the entire body wall (Sluys, 2019), therefore, the greater number of particles in the effluent treated by SG may have impaired the respiratory capacity of the planaria due to the adherence of the flakes to its body.

Bioassays of algal cell growth (*Raphidocelis subcapitata* and *Dunaliella tertiolecta*) demonstrated a decline in growth at 30 mg/L concentrations of fresh tannin water extracts (*Acacia* sp.) and immobilization of *Daphnia magna* at 100 to 300 mg/L. The authors of this work also concluded that water extract concentrations of fresh tannin water extracts resulted in the hormetic effect that corresponds to a positive stimulatory effect (De Nicola *et al*., 2004). As the present study was carried out in real effluent, it is not possible to rule out the hypothesis of a synergistic effect of the residual concentration of coagulants and the toxicity of the effluent itself.

Conclusion

Both the tannin-based coagulant (SG) and the ferrous aluminum sulfate (SF) were efficient in the formation of flocs and in the treatment of the evaluated effluent, however, in terms of ecotoxicity, SF showed 10% less toxicity than SG.

It is noteworthy that the tannin-based coagulant is biodegradable, which in the long term represents an advantage over the coagulant with aluminum salts. The accumulation of aluminum in the environment is a factor that needs attention and therefore its lower toxicity cannot be considered with a greater reduction of risks to the environment.

The biocoagulant showed interesting results for treatment systems that combine physical-chemical treatment (coagulation/floctation/flotation) with biological treatment, such as ponds. The physical-chemical treatment before the biological process would result in advantages such as odor reduction and detention time. The use of aluminum salts could harm the biological process due to the accumulation of aluminum salts due to the lower degradability of the sludge.

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