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Research paper

Ecotoxicological assessment of timber-industry wastewater based on bioassays conducted with lettuce seeds (*Lactuca sativa* L.)

Avaliação ecotoxicológica do efluente da indústria madeireira através de bioensaios com sementes de alface (Lactuca sativa L.)

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ABSTRACT: The Brazilian timber industry is one of the main activities in the national economy sector due to global increase in reforested wood consumption. Laminated veneer lumber stands out among wood-made products. The production of these materials accounts for generating liquid effluents with highly polluting features; therefore, it is essential acquiring ecotoxicological knowledge, which can be assessed through bioassays conducted with *Lactuca sativa* L seeds, which is the aim of the present study. Seed germination remained normal up to 75% effluent concentration. Approximately 20% of seeds presented inhibited germination under this condition. However, when 100% of effluent was used in the sample, 40% of seeds had their germination inhibited, likely due to chemical compounds, such as phenol, among other inorganic compounds. Seedling radicle recorded significant growth as effluent concentrations increased. Therefore, toxicological bioassays conducted with *L. sativa* seeds have proved to be effective in providing knowledge about potential environmental impacts in a simple, reliable and cost-effective manner.

Keywords: Environmental ecotoxicology; Bioindicators; Industrial wastewater.

RESUMO: *Devido ao crescimento mundial do consumo de madeira reflorestada, o Brasil tem a indústria madeireira como uma das principais atividades no setor da economia nacional. Dentre os produtos elaborados a partir da madeira estão os compensados laminados. A produção destes materiais é responsável pela geração de efluentes líquidos com características altamente poluidoras, portanto, torna-se imprescindível o seu conhecimento ecotoxicológico, o qual pode ser avaliado a partir de bioensaios utilizando sementes de Lactuca Sativa L., sendo este o objetivo do presente estudo. Quando avaliada a germinação das sementes, houve uma normalidade até que atingisse a concentração de 75%, nesta condição, cerca de 20% das sementes tiveram a germinação inibida, já quando utilizado 100% do efluente na amostra, foram inibidas 40% das sementes, tendo como possíveis responsáveis para tal, a presença de compostos químicos como o fenol e outros compostos inorgânicos. Quando avaliado o crescimento da radícula da plântula em contato com as diferentes concentrações de efluente, nota-se um acentuado crescimento em função do aumento da concentração. Portanto, os bioensaios toxicológicos utilizando sementes de Lactuca Sativa mostraram-se eficientes na obtenção do conhecimento dos possíveis impactos ambientais de forma simples, confiável e barata.*

Palavras-chave*: Ecotoxicologia ambiental; Bioindicadores; Efluentes industriais.*

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INTRODUCTION

Timber consumption worldwide and the large Brazilian territory available for reforested areas turns the national timber industry into one of the country's main economic activities, mostly in Southern states, such as Paraná and Santa Catarina (ABIMCI, 2013). Plywood manufacturing from Pinus logs, which has major influence on the important role played by this activity type in the Brazilian economic growth, stands out among production processes resulting from the timber industry. Most timber companies have some kind of environmental impact due to the generation of solid waste, whose inadequate management ends up producing liquid and gaseous effluents. Liquid effluents discharged in the environment without proper treatment can reach the soil, surface water and groundwater; consequently, they can affect local biodiversity.

According to some scholars, liquid effluents deriving from the timber industry, mainly the ones exclusively used in plywood production purposes, have great polluting potential because they have high concentrations of organic and inorganic matter, phenols, suspended and dissolved solids, as well as of phosphorus and its prevalent neutral or slightly acidic pH (TAYLOR *et al*., 1996; TAYLOR; CARMICHAEL, 2003; WOODHOUSE; DUFF, 2004; ZENAITIS *et al.*, 2002, HEDMARK, 2009). Improper disposal of this effluent type in nature can change watercolor, as well as increase its turbidity and the silting up of receiving bodies due to the presence of solids in them and to the eutrophication of receiving watercourses. Consequently, it can lead to the death of fish, among other organisms living in aquatic ecosystems. In addition, given its lipophilic nature, phenol can bioaccumulate along the trophic chain and reach humans.

Toxicity assessments can help preventing and avoiding several environmental impacts associated with effluents, since toxicology is the science focused on investigating negative and harmful effects generated from interactions between chemical substances and living organisms in the environment, under specific exposure conditions, based on knowledge from different fields, such as biochemistry, biology, chemistry, genetics, mathematics, medicine, pharmacology, physiology and physics (OGA *et al*., 2008; KLAASSEN; WATKINS III, 2012; SISINNO; OLIVEIRA-FILHO, 2013).This assessment aims at understanding effluents' behavior in contact with the environment, through bioassays conducted with bioindicators, such as *Lactuca sativa* L., which is popularly known as curly lettuce. This species stands out among those used to assess the phytotoxicity of a given compound, or of a set of them, through the inhibition of seed germination, as well as of radicle and hypocotyl elongation, at primary seedling stage (SOBRERO; RONCO, 2008).

These toxicological bioassays provide several results in a relatively fast, low-cost, simple and reliable manner, to help reducing environmental impacts caused by liquid effluents generated by the industrial sector, as well as better understanding their toxic potential. Therefore, the aim of the current study was to assess the toxicity of liquid effluent deriving from plywood production processes adopted by the timber industry, based on bioassays conducted with lettuce seeds (*L. sativa*), to help better understanding likely environmental impacts generated by this effluent type.

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MATERIAL AND METHODS

Effluent featuring

The liquid effluent used in the current research was collected from a timber company operating in Southern Brazil, most specifically in Paraná State, whose main commercial activity lies on laminated plywood manufacturing. The effluent was collected after solids' screening and sedimentation in primary decanter at the company's treatment plant. It was featured based on standard procedures described in the Standard Methods for the Examination of Water and Wastewater, 23rd edition, (APHA, 2017), in compliance with parameters shown in **Table 1**.

Samples were filtered through 0.45 mm membranes to remove solids from the liquid medium and to get the soluble fraction, in order to perform COD, phenolic compound and true color analyses.

Tabela 1. Parâmetros para caracterização do efluente e sua metodologia analítica empregada.

Parameters	Methodology used
Soluble chemical oxygen demand (COD)	Spectrophotometry - 5220D
Phenolic compounds	Spectrophotometry - 5530D
Apparent color	Spectrophotometry - 2120C
True color	Spectrophotometry - 2120C
Turbidity	Nephelometry - 2130B
Total solids	Gravimetry - 2540B

Source: APHA *et al*., 2012.

Assessing *Lactuca sativa* L. seeds' quality and germination

Before conducting toxicity tests with *L. sativa* seeds, one must ensure that their quality is approved, because seed exposed to different aqueous samples can show germination and growth inhibition (SOARES, 2000), and it can affect results observed for the investigated samples. Therefore, a negative control test was carried out in the absence of contaminants to assess the germination of the investigated batch.

Thirty-six seeds were distributed in two Petri dishes (18 seeds, each), along with Germitest filter paper. Then, distilled water was applied to the filter paper until it got fully wet. Subsequently, Petri dishes were sealed with plastic wrap and placed in natural convection oven under controlled temperature conditions (24ºC) for 7 days. The positive control test has followed the same protocol, but 1% zinc sulphate solution was used instead of distilled water to humidify the medium, as recommended by Machado *et al.* (2009).

Ecotoxicological bioassays

Dilutions of 1%, 5%, 10%, 25%, 50%, 75% and 100% of effluent deriving from the

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laminated plywood manufacturing company were used to assess the toxicity of these doses in ecotoxicological bioassays. Tests were carried out in duplicate. In total, 252 seeds were split 18 seeds per plate covered with Germitest filter paper and 36 seed per assessed dilution - by following the methodology adapted from Sobrero and Ronco (2008). Then, effluent was added to the paper in each Petri dish, at pre-defined concentrations, until it got fully wet. Subsequently, each Petri dish was sealed with plastic film and placed in oven at 24ºC, for 7 days.

After this period was over, the number of germinated seeds on each dish was counted and seedling's radicle size was measured with a caliper. These procedures enabled analyzing the interference in seed germination and development processes generated by different concentrations of the investigated effluent concentrations.

Although the assessed effluent presents high solids, color and turbidity levels, bioassays conducted with *L. sativa* seeds do not require pre-treatment, since removing the material accounting for these high concentrations could interfere with and change the actual research results (SOBRERO; RONCO, 2008).

Data statistical treatment

The Free Trial version of STATISTICA 7 (2005) software was used for data statistical treatment purposes, for 15 days, to enable better interpreting the results. After the germinated seed counting and rootlet measurement procedures were over, Barlett, Shapiro-Wilk and Durbin-Watson tests were applied to assess data homoscedasticity, independence, and normality, respectively. Subsequently, analysis of variance (ANOVA), at 95% significance level, was carried out to compare mean values observed for the assessed parameters (seed germination and radicle growth) based on the investigated effluent concentrations. Then, Tukey HSD test was carried out, based on using $\alpha = 0.05$, to determine both the similarity and disparity between treatments.

Finally, regression analyses were carried out to investigate the correlation of seedling radicle length and germinated seed rates to the applied effluent concentrations.

RESULTS AND DISCUSSIONS

Featuring effluent deriving from the laminated plywood company

Values shown in **Table 2** were recorded for parameters used to feature the liquid effluent deriving from laminated plywood manufacturing process.

Table 2 shows high concentrations of all assessed parameters; phenol was well above the standard value set for effluents discharged into water bodies, according to CONAMA Resolution n. 430, from 2011. High concentration of soluble chemical oxygen demand was directly linked to effluent's organic load. This finding has indicated the effluent's high polluting potential due to the organic material found in it. It is so, because this organic material accounts for several harmful impacts on water bodies, mainly for dissolved oxygen decay in the environment due to aerobic degradation. High phenol concentrations can lead to this compound bioaccumulation and affect the entire trophic chain, since this compound type presents low biodegradability and high hydrophobicity levels. In other words, it has greater affinity with fatty tissues and, therefore, it remains in

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the environment for longer and can even reach humans (HARNLY; BHAGWAT; LIN., 2007).

Table 2. Characterization of the liquid effluent generated from the production of laminated plywood.

Parameters	Valores médios
Soluble chemical oxygen demand (mg/L)	1067 ± 10.07
Phenol (mg/L)	27.43 ± 0.8
Apparent color (uC)	3450 ± 5.8
True color (uC)	307 ± 3.4
Turbidity (uT)	331 ± 2.16
Total solids (g/L)	4.73 ± 0.43

The high color and turbidity levels were mainly associated with colloidal, dissolved and suspended solids found in the effluent. These factors interfere with the passage of light. Thus, they impair plant photosynthesis and environment oxygenation - the deposition of these solids can lead to siltation in receiving water bodies. This finding has evidenced the high polluting potential of the investigated effluent and emphasized the need of better understanding likely negative environmental impacts generated by this wastewater type.

Positive and negative seed control tests

Based on the negative seed control test carried out with Germitest paper moistened with distilled water, 100% of seeds have germinated and the radicle's mean length reached 4.1 cm. This finding has evidenced reliable results based on the adopted batch of seeds, as well as lack seed-quality influence on the observed results. None of the analyzed seeds has germinated in the positive control test. As expected, 1% zinc sulphate was capable of inhibiting seed germination and development. Therefore, these analyses have validated results observed in the study carried out later on with different concentrations of the investigated effluent.

Ecotoxicological bioassays conducted with effluent deriving from the laminated plywood industry

Seed germination assessment

Seed germination rate was calculated based on counting *L. sativa* seeds presenting radicle length longer than 1 mm, which is the length set for germinated seed by Romero *et al.* (2014) Results are shown in **Figure 1**.

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Figure 1. Seed germination percentage as a function of the effluent concentrations applied.

Seed germination behavior remained normal until the sample was subjected to 75% effluent concentration, when only 80% of seeds have germinated. Reduced germination may be closely associated with some chemical compounds found in the effluent, which led to 20% seed inhibition. It is so, because high chemical oxygen demand value is linked to organic material content, as well as to some specific compounds released by Pinnus log-cooking processes, such as lignin, among other resins. The decrease observed in seed germination rate was even higher at 100% effluent concentration, since only 60% of seeds have germinated and 40% of them did not behave in any way. There statistically significant difference in seed germination between effluent concentrations of 60% and 100% ($p<0.05$); this difference was also significant in comparison to values recorded for other concentrations, which did not differ from each other, based on the Tukey HSD test results shown in **Table 3**.

Another point to be taken into consideration as likely cause for seed germination drop lies on the high phenol concentration found in the investigated effluent. Effluent concentration of 100%, for example, presented mean phenol concentration of 27.42 mg/L, which is considered extremely high, since0.5 mg/L is the standard effluent discharge concentration allowed by the relevant legislation (CONAMA Resolution n. 430/2011). Therefore, these high effluent levels could inhibit the germination of some seeds, since phenol is a highly-toxic compound. It is worth emphasizing that phenol can also be found present in both the root system and structure of seedlings that managed to germinate, since this compound shows high affinity to organisms (lipophilic) and low affinity to water (hydrophobic), a fact that makes it accountable for several bioaccumulation cases at different trophic chain levels.

Several toxicity studies have been carried out based on using species *L. sativa* as bioindicator. Santos *et al.* (2014) investigated the influence of a commercial sample presenting the food coloring “azorrubine” and observed negative effect on plant growth, which directly affected initial seedling development. Macena *et al.* (2017) assessed the phytotoxicity of the compound “atrazine” in seeds belonging to the same lettuce species. Based on their results, the authors got to the conclusion that the investigated compound presented strong toxicity potential over the analyzed seeds, as well as exponential effects

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on their germination. Gryczak *et al.* (2018) used *L. sativa* seeds to assess the toxicity of solubilized effluent deriving from construction waste and did not find any toxicity whatsoever, a fact that corroborated the benef of reusing this wastewater type. As previously mentioned, several studies have been carried out to assess the ecotoxicity of a wide range of effluents based on this methodology, since it is easy to apply and develop, and provides good and representative results at low cost. However, studies adopting this methodology type to assess effluents deriving from the processes to manufacture laminated plywood deriving from pine-log cooking remain scarce or non-existent in the literute.

Radicle length assessment

Results observed in bioassays carried out with different concentrations of effluent deriving from laminated plywood processes are shown in **Table 3**.

Table 3. Evaluation of the initial development of the seedling from the different concentrations of effluent.

Percentage of effluent in the sample (%)	Average radicle length (cm)	Germination percentage (%)
1	4.12 f	100 a
5	4.64 e	100 a
10	4.72 e	100 a
25	4.85 d	100 a
50	5.12 c	100 a
75	6.92 b	80 b
100	7.56 a	60 c

**Means followed by the same letter do not differ statistically by Tukey's test at 5% significance.*

Mean radicle length recorded statistically significant difference in growth as effluent concentrations increased ($p<0.05$). In other words, the higher the effluent concentration in the sample, the higher the seedling radicle growth rate. This finding was confirmed through Tukey HSD test, which only showedsimilarity between effluent concentrations of 5% and 10%, which differed from all other concentrations, which, in their turn, also differed from each other, as shown in **Table 3**. The straight correlation between radicle growth and effluent concentration increase was clear, as shown in **Figure 2**.

The high R^2 value (0.9261) observed in the scatter plot of radicle growth data was clearly correlated to increase in effluent concentrations. This assessment type is valid because it can analyze low contaminant concentrations that are not capable of inhibiting seed germination. However, seedling growth can either slow down or speed up at the primary stage, depending on the effluent compounds. This factor turns *L. sativa* into a sub-lethal indicator due to its sensitivity to assess contaminants in plants (SOBRERO; RONCO, 2008).

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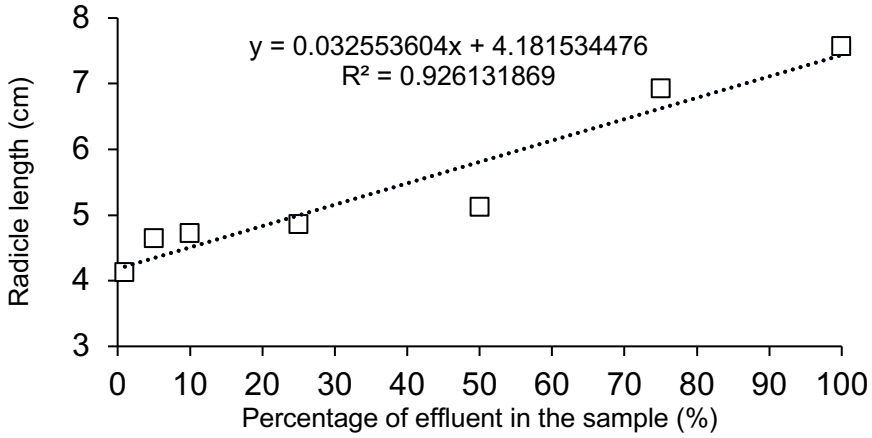
Figure 2. Radicle length as a function of the dosages evaluated.



Radicle growth acceleration may be closely linked to the amount of organic matter in the effluent, since it presents high chemical oxygen demand and total solids concentrations and enables better conditions for initial seedling development. According to the literature, this effluent type also has high phosphorus concentrations, which work as nutrient for such growth. The high organic material concentration favoring radicle growth is also accountable for decreasing dissolved oxygen rates in water bodies. Organic matter tends to be degraded by aerobic bacteria found in the environment. It is so, because, these bacteria consume the oxygen in the environment and use it for organic matter degradation purposes. This process favors the emergence of an anaerobic environment that accounts for the death of fish, among other aerobic organisms living in it (ALVIM *et al.,* 2011).

CONCLUSIONS

Effluent deriving from laminated plywood manufacturing in the timber industry has direct influence on radicle growth of *L. sativa* seeds, likely due to high soluble chemical oxygen demand concentrations found in it. This factor indicates higher organic matter and nutrient availability that may have favored radicle growth. These compounds account for several environmental impacts on water bodies when they are discharged in them without proper treatment.

In addition, germination analyses have shown that the effluent has the potential to inhibit the germination of most of the analyzed seeds when higher concentrations of it are used. It likely happens due to the presence of chemical compounds, such as phenol, in it. This inhibition can be observed in different species found in the environment, a fact that directly affects local biodiversity.

Therefore, ecotoxicity bioassays conducted with species *L. sativa* are of paramount importance to assess environmental toxicology, given their economic viability, speed, reliability and easy-to-apply methodology that, in its turn, enables predicting the toxic potential of effluents, such as the one deriving form laminated plywood manufacturing processes.

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