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Toxicity Assessment of Tanning Effluents Treated via Electrocoagulation and Ozonation Using a Bioassay with *Lactuca sativa* L.

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ABSTRACT

In this study, the aim is to assess the toxicity of tannery wastewater treated with electrocoagulation and ozonation to determine the suitability of the approach for application, while maintaining the environmental quality of receiving water bodies and/or sewer systems. For this, an electrocoagulation reactor and an ozonation tank were built considering current intensity (I), treatment time (T), and ozone concentration O_3 as operating factors. Acute toxicity tests were conducted using *Lactuca sativa* L. lettuce seeds for the raw sample (MI), sample treated with electrocoagulation (EC), and sample treated with EC and ozonation (EC + OZ). The toxicity parameters assessed in this study were the absolute germination (AG), germination index (GI), and average inhibition concentration (EC50). The electrocoagulation reactor achieved 92% removal efficiency for total suspended solid (TSS) and 10% removal efficiency for chemical oxygen demand (COD) with a current intensity of 7A and a treatment time of 30 min. In addition, the COD was further reduced in the ozonation tank by 18% with an ozone dosage of 10 g/h and a contact time of 30 min. Despite these treatments, EC50 values indicated acute toxicity in all three samples. The ANOVA analysis (p value of 0.05) revealed no significant differences between the GI values for the three samples, suggesting that toxicity did not decrease substantially, despite treatment. This is attributed to the incomplete removal of the pollutant load, expressed as COD, and formation of recalcitrant and toxic compounds during treatment processes. This work demonstrates the importance of including the "toxicity" variable in the assessment of treatments to conduct them in an integral way and preserve the environmental quality of receiving water bodies.

Keywords: electrocoagulation, toxicity, ozonation, *Lactuca sativa* L.

INTRODUCTION

In the tanning industry, animal skins from diverse sources are transformed into leather. Tanning renders leather imperishable, improves its resistance, and turns it into a material that provides protection, comfort, and functionality to the. Leather is characterized using the properties of thermoregulation, permeability to sweat and gases, tensile and tear resistances, and elongation (Meyer, 2021; Sudha, 2009; Ahmed, 2021). Tanning is an extensive and complex process and comprises several substages in which byproducts of high environmental concern are produced. These byproducts are mainly solid waste and wastewater (Mwinyihija, 2010). In the soaking, fleshing, deliming, tanning, retanning, dyeing, and fatliquoring substeps, effluents of different compositions are produced depending on the chemical products used (Susanto et al., 2023; Kanagaraj et al., 2020).

In general, the effluents of this industry are characterized by high contents of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), ammonium, sulfides, chlorides, and chromium, a substance

used in the tanning process as basic chromium sulfate and is the most widely used tanning agent in the industry (Barra-Hinojosa et al., 2024).

Effluents generated by this industry are discharged into sewer systems or water bodies depending on their location. Currently, industrial wastewater is treated via chemical coagulation and flocculation; however, these processes do not ensure pollutant removal to the levels required by environmental regulations. Hence, there is an urgent need to implement alternative or complementary techniques that allow the complete removal of such pollutants.

Several studies have demonstrated that electrocoagulation is efficient in reducing various pollutants indicator parameters from tannery effluents such as BOD, COD, TSS, and chromium (Aguilar et al., 2019; Liu et al., 2018).

However, there is a precedent indicating that the use of electrocoagulation together with an advanced oxidation treatment with ozone (Barzegar et al., 2019) increases its efficiency, consuming even less energy (Liu et al., 2018). Several studies have combined the processes proposed in this study electrocoagulation and ozonation for treating tannery wastewater and various types of effluents. A study by (Hernández-Ortega et al. 2010) revealed that the coupling of electrocoagulation and ozonation is useful as a complete treatment for the discharge of industrial effluents into municipal sewers. They were able to decrease the color and turbidity of wastewater by more than 90% and COD by more than 60%. In addition, (Asaithambi et al., 2016) compared the effectiveness of the ozonation, electrocoagulation, and ozone-assisted electrocoagulation processes for the removal of pollutants from distillation industry effluents. Furthermore, using an integrated process (ozonation and electrocoagulation), (García-Morales, 2013) achieved 65% color removal, 76% turbidity elimination, and 37% COD reduction. In addition, this study indicated that synergies associated with the use of both processes increased the removal of color, COD, and turbidity compared with the results obtained when individual treatments are applied. In another study by (Preethi, 2009), ozone was used in the treatment of tannery effluents, and their results indicated that the percentage of COD removal gradually increased with the process time, achieving a maximum 70% COD removal after approximately 90 min of ozonation. Electrocoagulation (EC) is an electrochemical process in which direct current is applied to dissolve aluminum, iron, or other metals used as sacrificial anodes immersed in pollutant wastewater (Shahedi, 2020). Electrodissolution poses the issues of an increase in the content of ions in the aqueous phase or their complexed species with the OH content depending on the used sacrificial anode and pH conditions (Kamaraj R, 2015; Shahedi, 2020). These species act as destabilizing agents or coagulants and lead to the separation of pollutants from the aqueous phase (Shahedi, 2020). These coagulants allow the formation of flocs that rise to the surface by a flotation process, while the other floccules precipitate (Esfandyari et al., 2019; Aguilar et al., 2020). This process is considered an effective method due to the increased adsorption of OH^- on the mineral surface in situ compared with chemical treatments in which metal hydroxides are used as coagulants (Peralta, 2014). As flocs produced by EC are relatively large, they are more stable and contain less bound water; hence, they could be easily separated via filtration (Zaroual, 2006; Shahedi, 2020).

Main reactions that occur in the electrodes are as follows (Elabbas, 2016):

- At the aluminum anode:

$$Al \rightarrow Al^{3+} + 3e^- \quad (1)$$

- At the aluminum cathode:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (2)$$

In addition, in the presence of chloride ions, the aluminum cathode can dissolve and generate hydrogen.

$$Al + 3H_2O \rightarrow Al^{3+} + 3OH^- + 3/2H_2 \quad (3)$$

$$Al^{3+} + 2OH^- \rightarrow Al(OH)_3 \quad (4)$$

The EC process has advantages compared with conventional treatments. Sludge is produced in the EC process in comparison with chemical coagulation (Koyuncu and Arıman, 2020). Furthermore, EC does not use chemical products, has a simpler operation and maintenance, takes up less space, and can be installed by modules.

Advanced oxidation process (AOP) are technologies based predominantly on the production of hydroxyl radicals. The AOP include the use of UV, UV/H_2O_2, UV/($3O_3$/H_2O_2, and O_3/H_2O_2/UV, Fenton process, and photo-Fenton and sonochemical processes (Rodriguez-Narvaez O, 2017; Lanzetta, 2023). In recent years, the interest in the AOP has increased due to its ability to degrade recalcitrant components without generating a secondary waste stream (Dewil, 2017; Lanzetta, 2023). Ozone, being a strong oxidant, is widely

applied for water disinfection and organic pollutant degradation (Liu et al., 2021). Meanwhile, ozone-based processes of advanced oxidation are effective and easy to handle for treating recalcitrant pollutants in wastewater without generating secondary waste (Joseph et al., 2021). Furthermore, ozonation is commonly used as a pre- and post-treatment along with biological processes (Lotito et al., 2012). Being a powerful oxidizer, ozone is used for the removal of color, odor, phenolic compounds, and recalcitrant organic pollutants from industrial wastewater and leachates. In addition, it has the advantage of not producing sludge or requiring additional chemical products (Saranya, 2020; Joshi and Gogate, 2019). The use of ozone is even more important in the treatment of industrial wastewater because complex molecules derived from anthropogenic pollutants are rarely attacked by microorganisms in biological processes (Lanzetta, 2023). However, the use of ozonation is not considered an economically viable process for treating industrial effluents due to the requirements of high doses of ozone for the complete mineralization of organic pollutants and additional treatment methods for the remediation of inorganic residues left by ozonation, such as inorganic carbon, ammonium ions, nitrates, phosphates, and other inorganic metals in effluents (Saranya, 2020; Schroeder et al., 2011). This can be solved by integrating technologies that complement the treatment and allow for a reduction in costs. Because O_3 generation is expensive, there is a consensus that O_3 is an excellent reagent for mineralizing the persistent COD. However, in the first place, it should be used particularly for the conversion of the refractory organic matter into biodegradable compounds before employing it for conventional treatment. (Schrank et al., 2016; Lee et al. 2014).

Despite the benefits of the technologies described above, and in addition to assessing effluents treated physically, chemically and microbiologically, it is necessary to determine their level of toxicity. This is because this property of the effluent may be modified in a negative or positive way according to the treatment technique used, leading to physicochemical processes that cause the formation of new components, which may or may not result in toxicity. This implies that although the normalized pollutants are removed, others are generated that can affect the quality of the dumping source. Ecotoxicology studies toxic effects caused by different types of substances on the biotic or abiotic components of an ecosystem (Belden, 2020). For this, to understand and predict the effects of various substances, it uses as a tool study on living beings under real exposure conditions. These studies are called bioassays or toxicity tests (Belden, 2020).

In these tests, assessments are conducted on certain organisms (known as biological models or bioindicators) selected for their sensitivity to the presence of toxic substances (Belden, 2020). Effects can be assessed based on growth inhibition; morphological, physiological, or genetic changes; or mortality (Mwinyihija, 2010). Additionally, this tool has been applied for the assessment of environmental matrices, such as wastewater, as reported in a previous work of (Methneni et al., 2021). Here, the toxic effluents of the textile industry were assessed before and after the treatment using a series of bioassays with several test organisms (*Selenastrum capricornutum*, *Vibrio fischeri*, *Daphnia magna*, and *Lepidium sativum*) for identifying the high toxicity of effluents arising from the presence of metal contents and persistent dyes even after treatment (Methneni et al., 2021).

Among the most widely applied bioassays is lettuce seeds (*Lactuca sativa L.*), which is considered a static acute toxicity test with 120 h of exposure, and is used for analyzing the phytotoxic effects of assessed samples. The determined effects inhibit seed germination and seedling development, which are measured based on the elongation of their radicles with respect to a sample exposed to a standard solution with nutrients (negative control) and to a solution containing a reference pollutant (positive control) (Castillo-Morales, 2004).

In a previous research, (Palacio et al., 2009) used bioassays on *Lactuca sativa* L. seeds to assess the toxicity of textile industry effluents treated via EC, identifying low toxicity in samples treated for 5 min compared with longer treatments. Similarly, (Manenti et al., 2015) found that least toxic effects were observed in a textile effluent 5–15 min after the application of the EC conditions, concluding that the increase in toxicity over time could be attributed to the formation of recalcitrant compounds during treatment (Manenti et al., 2015).

Jallouli et al., (2020) assessed the effect of raw tannery wastewater treated with EC and UV photolysis on inhibiting seed germination and the rooting and apical growth of *Hordeum Vulgare* seeds. The obtained results indicated a greater toxicity for untreated effluents than for treated ones.

Saranya and Shanthakumar, 2020 studied the toxicity of a tannery effluent treated via ozonation at different time intervals. The organism used for the estimation of toxicity was the marine microalgae *Nannochloropsis oculata*; moreover, the effect measured was microalgal cell density. The effluent collected during 10 to 30 min was reported not to favor the microalgal growth and imposed a toxic oxidative stress on microalgae due to (i) the lethal concentration of sulfides and other inorganic salts that act as inhibitors of the microalgal growth and (ii) organic compounds and their derivatives formed during ozonation. At longer duration of ozonation, microalgae acclimatization was observed, and at 90 min, a higher biomass concentration was recorded with respect to the control due to the reduced toxicity and high concentration of the preferred nutrient source NH_4+N and inorganic carbon.

The objective of this research work is to evaluate the toxicity contribution of electrocoagulation and ozonation as tannery wastewater treatments, in order to establish the suitability of their application in the tannery industry while maintaining the environmental quality of the receiving water bodies and/or sewage systems.

MATERIALS AND METHODS

Tannery wastewater

The tannery wastewater was supplied by the Center for Productive Innovation and Technological Transfer of Leather, Footwear, Textiles, Clothing and Related Industries (CITEccal Lima) and was obtained from a tanning process of beef hides using basic chromium sulfate as a tanner. This process was performed in the Pilot Tannery Plant of CITEccal Lima according to the formulation shown in Table 1.

The following commercial chemicals were used in the tanning process: wetting agent (UD-800), bactericide (Tensocide 85), soaking water softener (Neutragene PK), degreasing surfactant (Cletapon FU-100), organic acid (Unictan TC-400), fungicide (Tensocide EB), unhairing auxiliary agent (LA-X7), amino compounds (Ecolime PHB), chromium exhausting agent (Chymancrom AGP), and proteolytic enzymes (Macerant 1500).

Amount – is the mass in (w/w %) regarding the skin weight. "Duration" is the time spent in each stage. Observations – describes controls required in the process

Wastewater was obtained as a sample comprising effluents from the soaking, peeling, deliming, and tanning operations. The amount used for each operation was proportional to the volume of effluent generated.

Raw wastewater (MI) was characterized based on its TSS content, turbidity, pH conductivity, COD, and BOD. For measuring pH, conductivity, and temperature, an Oakton PCS 35 multiparameter was used, and TSS was quantified using a DR900 Hach portable colorimeter. The method used for the determination of BOD was SMEWW-APHA-AWWA-WEF Part 5210 B;23rd Ed: 2017. BOD: 5-day BOD test; and for COD, SMEW SMEWW-APHA-AWWA-WEF Part 5220 D: 23rd Ed: 2017 was employed. COD, closed reflux, colorimetric method. 2017W-APHA-AWWA-WEF Part 4500-NH_3 D, 24th Ed. 2023. Samples were collected and stored according to the requirements of each test method. Bioassay samples were placed in plastic containers and stored at a temperature below 5 °C for no more than 48 h until analysis.

EC reactor: Ozonation

The EC reactor was designed with dimensions of 16 cm, by 16 cm, and by 22 cm in length, width, and height, respectively. Eight aluminum electrodes working as an anode and cathode were used, with a dimension of 10 cm in width, 10 cm in length, and an area of 100 cm^2. An adjustable power source with a capacity of 50 A was used for supplying current. Perforated hose diffusers were installed in the ozonation tank for supplying ozone to the water. This ozone was generated with an ozonizer that can supply an ozone concentration of up to 10 gr/h. A built-in pump was used to feed air to the ozone generation chamber, where transformation of O_2 to O_3 occurs (Fig. 1).

It comprised two stages: the removal of TSS in the electrocoagulation reactor and the reduction of the organic load to obtain a better quality of treated water for toxicity tests.

Acute toxicity test with *Lactuca sativa*

For the bioassay, light-green, long romaine lettuce seeds of the BATLLE S.A. brand with a high germination percentage were used. Reagents used for the development of the assay were of analytical grade: sodium hydrogen carbonate (reagent grade, ACS, ISO), potassium chloride

Table 1. Tanning process. Product column provides the description of the product used in each stage.

Process	Product	Amount (w/w %)	Duration (min)	Observations
Pre-soaking	Wetting agent	0.3	120	Achieve skin moisturization.
	Bactericide	0.2		
	Water	250		
Soaking	Wetting agent	0.3	30	Finished, let the skins in the bath rest for 20–24 hours. Once finished, drain the drum and wash with tap water.
	Bactericide	0.2		
	Lime	0.2		
	Soaking water softener	0.5		
	Water	200		
Unhairing	Amino compounds	0.5	20	Once finished, drain the drum and wash with tap water.
	Wetting agent	0.2	45	
	Unhairing auxiliary agent	0.1		
	Lime	1.0		
	Sodium sulfide	0.5	30	
	Unhairing auxiliary agent	0.1		
	Degreasing surfactant	0.1		
	Sodium sulfide	1.0	45	
	Lime	0.5		
	Amino compounds	0.2	60	
	Sodium sulfide	0.5		
	Lime	2.0		
Deliming	Ammonium sulfate	0.3	10	Once finished, drain the drum.
	Water	300		
	Degreasing surfactant	0.1	20	
	Ammonium sulfate	1.0	90	
	Sodium bisulfite	0.6		Keep the bath for the bating.
	Water (28 °C)	20		
Bating	Proteolytic enzymes	0.5	50	
	Wetting agent	0.2		
Washing	Water	100	40	Once finished, drain the drum.
	Wetting agent	0.5		
	Water	300	–	Wash carefully until there are no foam residues.
Pickling	Ground salt	10	15	Once finished, control the pH with bromocresol green indicator, final pH must be in the range of 3–4. Keep the bath for tanning.
	Water (28 °C)	100		
	Organic acid	1.0	30	
	Organic acid	2.0	120	
Tanning	Basic chromium sulfate	3.5	30	
	Basic chromium sulfate	3.5	30	
	Chromium exhausting agent	0.5		
	Sodium formate	0.5		
	Cationic oil	0.5		
	Sodium bicarbonate	1.2	120	The sodium bicarbonate must be diluted in water and added in 4 equal parts every 30 minutes. Once finished let the drum operating for 8 hours.
	Fungicide	0.2	60	Once finished, drain the drum.
	Water (50 °C)	30		

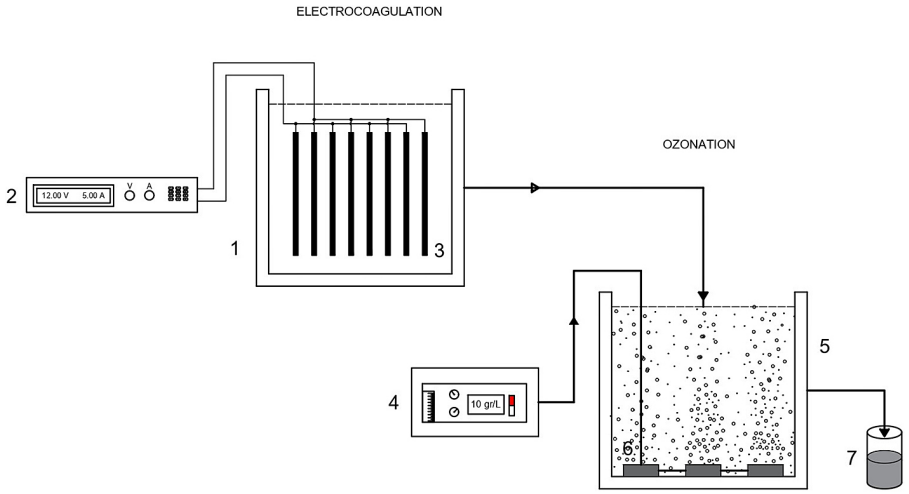

Figure 1. Schematic of the integrated treatment system. (1) Electrocoagulation reactor (2) Power source (3) Aluminum electrodes (4) Ozonizer (5) Ozonation tank (6) Diffusers (7) Reception tank

(reagent grade), and magnesium sulfate anhydrous (extra pure) were purchased from Scharlau®; calcium sulfate dihydrate precipitated and zinc sulfate heptahydrate for analysis were procured from EMSURE®.

The bioassay design included a negative control, a positive control and the effluent samples untreated, treated only with electrocoagulation and treated by electrocoagulation and ozone. The negative control or reconstituted hard water was prepared by dissolving 2.4 g $MgSO_4$, 3.84 g $NaHCO_3$, 0.16 g KCl, and 2.4 g $CaSO_4$ in 20 L of distilled water (SECOFI, 1995). The positive control comprises five zinc sulfate concentrations (50, 100, 150, 200, and 250 mg/L) prepared from a 500 mg/L $ZnSO_4$ solution in reconstituted water. Each of the effluent samples was diluted with reconstituted hard water in the following percentages: 1%, 3%, 10%, 30%, and 100%.

The lettuce seeds was sowed in petri dishes with a diameter of 10 cm for the negative and positive controls and effluent samples, and the process was triplicated. Herein, 20 seeds were placed per plate and 2 mL of each solution was added to petri dishes, according to the distribution shown in Figure 2 Subsequently, the petri dishes were placed in bags and incubated at 22 ± 2 °C in the dark for 120 h (Castillo-Morales, 2004). At the end of the exposure time, the root growth of each germinated seed was performed. Data processing was performed with Microsoft Excel Real Statistics add-in, and the average inhibition concentration (EC50) was calculated using a dose response curve by applying linear regression. The calculation of absolute germination (AG) and the germination index (GI) was performed according to Equations 5 and 6.

$$AG = \frac{N_{germ}}{N_{seed}} \tag{5}$$

$$GI = \frac{N_{germ}}{N_{cont}} \times \frac{RL_{germ}}{RL_{cont}} \tag{6}$$

where: N_{germ} = average amount of seeds germinated, N_{seed} = total amount of seeds, N_{cont} = amount of germinated seeds in the negative control, RL_{germ} = average amount of root lengthening in the sample, RL_{cont} = average root lengthening in the control.

The EC50 result was used to calculate the value of toxicity units according to the formula presented in the Equation 7. The classification of toxicity units was used as a criterion to determine the acute toxicity class to which the sample belongs, according to the classification presented in the Table 2 (Persoone, 2003).

$$TU = EC_{50} \times 100 \tag{7}$$

where: *TU* indicates toxicity units.

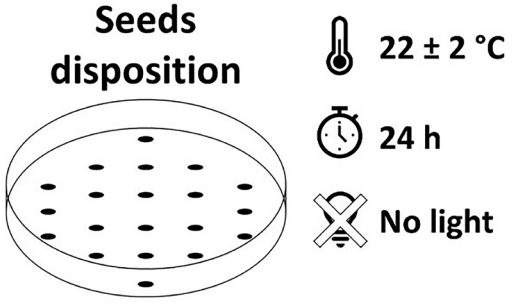

Figure 2. Seed arrangement in a petri dish and the applied bioassay conditions

Table 2. Hazard classification system

TU	Class	Toxicity
<0.4	Class I	No acute toxicity
0.4 < TU < 1	Class II	Slight acute toxicity
1 < TU < 10	Class III	Acute toxicity
10 < TU < 100	Class IV	High acute toxicity
TU > 100	Class V	Very high acute toxicity

Statistical analysis

The statistical analysis of the GI results was performed by assessing significant differences between the types of effluents used via variance analysis (ANOVA). For this, the "lmtest" and "car" packages were applied to the R software version 4.3.2 (Core Team R, 2023).

RESULTS AND DISCUSSION

Characterization of effluents

Table 4 reveals the results for the characterization of MI. The values obtained exceed the maximum limits allowed by the national legislation; the allowed values are a value lower or equal to 30 mg/L for BOD, lower or equal to 50mg/L for COD, lower or equal to 30 mg/L for TSS, and lower or equal to 0.5 mg/L for total chromium. However, pH of the effluent was kept within the allowed range of 6–9.

The observed significant pollution originates from chemicals utilized in tanning and the inherent organic composition of the skin. Pretanning involves the use of various salts including sodium chloride, ammonium sulfate, sodium sulfide, lime, basic chromium sulfate; organic and inorganic acids; enzymes; and bactericides. Post-tanning procedures involve the application of deacidifiers, synthetic and natural retanning agents, synthetic and natural oils, surfactants, dyes, and additional chemical aids (Hansen et al.,2020).

The values determined in the sample under study are shown in Table 3 and are within the range of values reported by other studies. Hence, we have the values of pH within the range of 7.0 and 8.49, TSS between 194 and 6000 mg/L, BOD between 92 and 1300 mg/L, COD between 322 and 9000 mg/L, turbidity between 332 and 1810 mg/L conductivity between 4160 and 50000 mg/L, and chromium between 5.7 and 123.18 mg/L.

Effluents treated with electrocoagulation and ozonation

In the first stage, the raw tannery wastewater was treated in the EC reactor using aluminum electrodes considering the current intensity and treatment time as operating parameters, and the removal efficiency of TSS was assessed to improve the quality of the effluent. The TSS concentration was reduced from 980 mg/L to 75 mg/L, yielding a removal efficiency of 92% at a current intensity of 7A and a treatment time of 30 min. EC exhibits better performance for the removal of TSS. However, it does not show such a high efficiency for COD, reducing the initial concentration of COD in MI from 2961 mg/L to 2643 mg/L, which corresponds to a removal efficiency of 10.7%. This low yield may be due to the formation of recalcitrant

Table 3. Physicochemical analysis of the effluent

Parameter	Value	Values obtained in other studies
Total suspended solids (mg/L)	980	na[a], 1790[b], 194 ± 23.5[c], 1578[d], 6000 ± 600[e], na[f]
Chemical oxygen demand (mg/L)	2961	2100+/30[a], 942[b], 322 ± 28.6[c], 5308.4[d], 14,000 ± 1400[e], 6000–9000[f]
Biochemical oxygen demand (mg/L)	561	na[a], 92[b], 160 ± 15.8[c], 1952.5[d], 1800 ± 180[e], 1120–2300[f]
Turbidity (mg/L)	1300	na[a], 332NTU[b], na[c], 1810NTU[d], > 999[e], 220–440NTU[f]
pH	7.28	8.0+/−0.1[a] ,7.1[b], 8.49 ± 0.2[c], 8.36[d], 7 ± 0.7[e], H 7.2–7[f]
Conductivity (µS/cm)	15100	21100+/−100[a], 9600[b], 4160 ± 70[c], 10430[d], 50000 ± 500[e], 5500–9000[f]
Total chromium (mg/L)	89.52	37+/−0.9[a], na[b], 5.7 ± 0.2[c], 123.18[d], 25 ± 2.5[e], na[f]

Note: Na – not available. [a] Tesfaye (2020), [b] Zakmout (2020), [c] Bharagava (2018), [d] Aguilar (2019), [e] Villalobos (2020), [f] Züleyha (2023).

intermediates during the electrochemical process (Manenti et al., 2015). This value is lower than that reported by (Apaydin et al., 2009), achieving a COD removal efficiency of 46%. Meanwhile, (Espinoza et al., 2009) reported an average efficiency of 50% by employing a treatment duration of 30–60 min. In EC treatments, the removal efficiency of COD could be approximately 40%, (Manenti et al., 2015; Gilpavas et al., 2020). This value could be attributed to the inadequate generation of flocs to remove mainly organic pollutants, despite there being a high removal of TSS; hence, there is a need to supplement the treatment with oxidizing agents such as ozone.

In the second stage, water treated with EC was subjected to treatment in the ozonation tank, wherein ozone was supplied at a concentration of 10 gr/L and a contact time of 30 min was provided, to degrade the organic matter expressed as COD. In the ozonation tank, the concentration of COD decreased to a value of 2165 mg/L, indicating a further removal of 18%. In addition, thanks to the ozonation process, it was possible to reduce the concentration of TSS up to a value of 12 mg/L, thereby achieving a total removal efficiency of 98% for TSS. This is consistent with the results reported by (Laconi et al., 2009), who achieved a TSS removal of 85%. Meanwhile, (Sekar, 2008) studied the effect of ozone concentration on the elimination of COD and TSS. They reported a maximum COD reduction of 47% from a concentration of 7.2 ppm; however, for TSS, only a small reduction from 11% to 10% was achieved. There are other studies where the two processes are combined (electrocoagulation - ozone), where Hernández-Ortega et al., 2010, reports a COD reduction of 60% and over 90% in industrial wastewater. Another study by (Asaithambi et al, 2012), indicates that the hybrid technique was more effective than electrocoagulation and ozonation separately, and achieves a COD removal rate of 45% to 92%.

Acute toxicity test with Lactuca sativa

The results of AG, GI, EC50 and toxicity units obtained for each sample assessed with the bioassay are presented in Table 4. The toxicity classification according to the toxicity units obtained by EC50 indicates that the three samples are within the classification of "Class III – acute toxicity" (Persoone, 2003). Meanwhile, the obtained GI values are consistent with effluent concentrations used in the bioassay, where a decrease in the GI is identified with increasing sample concentration. Figure 3 shows the results of the GI obtained at different concentrations and for each sample.

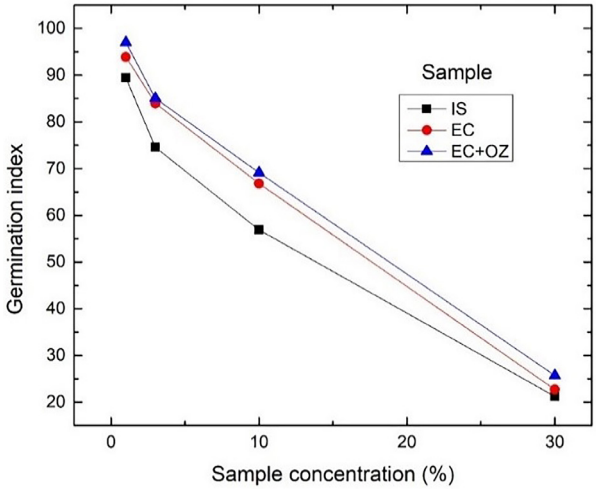

Figure 3. Germination index per concentration and sample.
GI is expressed as percentage of its values on Table 4

Table 4. Results of toxicity acute bioassay on *Lactuca sativa* seeds

Samples	1		3		10		30		100		EC50 (%)	Toxic units
	AG	GI	AG	GI	AG	GI	AG	GI	AG	GI		
MI	1.00	0.89	0.98	0.75	0.93	0.57	0.65	0.21	0	0	19.34	5.17
EC	0.88	0.94	0.90	0.84	1.00	0.67	0.93	0.23	0	0	19.49	5.13
EC + OZ	1.00	0.97	0.98	0.85	1.00	0.69	0.88	0.26	0	0	20.12	4.97

According to the qualitative classification of toxicity for the GI, samples at a dilution of 1% can be considered as nonphytotoxic. Furthermore, a concentration of 3% for samples treated with EC and EC + OZ is considered to be nonphytotoxic, while MI has a level considered as moderately phytotoxic. As for the concentration of 10%, EC – and EC + OZ – treated samples are considered to be moderately phytotoxic and MI is assumed as a phytotoxic. All samples at a concentration of 30% are classified as phytotoxic. Furthermore, samples at a concentration of 100%, which show the complete inhibition of germination, are classified as very phytotoxic.

The analysis of the qualitative classification of toxicity for the GI seems to indicate that, at lower dilution, the EC – and EC + OZ – treated samples are less toxic than MI; therefore, an analysis of ANOVA was applied to identify significant differences between effluent samples. The analysis considered the GI value as a dependent variable and concentration and type of effluent as independent variables. The results are listed in Table 5. Based on the ANOVA result, a coefficient of determination of 0.9179 and an adjusted coefficient of 0.8494 were obtained, and the model fulfilled the assumptions of normality (0.0909), hoscedasticity (0.1129), and independence of errors (0.2419). The concentration effect on the GI (< 0.05) was demonstrated, while the type of sample showed no significance; therefore, there are no significant differences between the results of the GI for different assessed effluent samples.

Although the pollution load of the EC – and EC + OZ – treated effluents reduced with respect to MI, mainly in terms of TSS, no significant variation exists in toxicity. In the case of EC, even with the removal of oxidizable organic matter, nitrogen-containing compounds and oxidizable salts used in the tanning process (Sameh et al., 2020) and soluble recalcitrant substances were formed (Manenti et al., 2015), which will maintain the levels of DQO. Note that these COD levels arise from the large number of chemical inputs used in the tanning process.

Research has shown that the elevated levels of COD can result in toxic conditions in aquatic environments; hence, a notable decrease in toxicity will not be observed in treated samples. Similarly, studies have demonstrated that longer EC durations lead to heightened toxicity in treated effluents, observing a marked contrast between treatment times of 5 and 30 min (Manenti et al., 2015). In this study, toxicity assessments were conducted under the optimal treatment conditions of 30 min EC, potentially elevating the toxicity of the treated effluent to the same levels as the untreated sample. However, other studies have reported that, at 90 minute durations, effluent toxicity decreases notably (De Pauli et al., 2018), suggesting a more substantial removal of pollutants over time.

Furthermore, EC can generate ammonia through the electrochemical conversion of nitrates to nitrites, which subsequently transform into ammonia at the cathode (De Pauli et al., 2018). During the tanning process of skins, ammonia nitrogen also originates at the liming stage. The toxicity of the EC + OZ -treated sample is associated with an indirect oxidation reaction mechanism with ozone, which give rises to reactive oxygen species, such as superoxide anion (O_2), hydroperoxyl radical (HO_2), ozonide anion (O_3), and hydrogen trioxide radical HO_3 (Saranya et al., 2020). These species will maintain toxicity levels, despite the removal of 28% COD, as they are shown to be associated with toxic effects on organisms (Yan ZQ et al., 2015).

Table 5. Analysis of variance table response germination index

Parameter	Sum. Sq.	Df.	F-value	Pr (>F)
x1: Concentration	7729.3	1	65.642	0.0001896 ***
x2: Sample	165.2	2	0.7013	0.5324578
x1:x2	1.9	2	0.0081	0.9919753
Residuals	706.5	6	-	-
Multiple R-squared: 0.9179		Adjusted R-squared: 0.8494		
Model assumptions				
Normal distribution		Shapiro–Wilk normality test		p-value = 0.0909
Homoscedasticity		Studentized Breusch–Pagan test		p-value = 0.1129
Independence of errors		Breusch–Godfrey test		p-value = 0.2419

With the aim of removing the residual contaminant load, various studies suggest that physicochemical treatments should be complemented with biological treatments in such a way that the residual organic substances are degraded (Moktadir et al., 2024).

The data obtained in this research constitutes preliminary evidence of the toxicity contributed by these treatments. To achieve a better approximation, it is necessary to evaluate toxicity in various bioindicator species and select as parameters those values that protect 95% of the species. On the other hand, when technologies are scaled up to a real level, biological monitoring of the organisms present in water bodies is crucial for controlling the balance in ecosystems (Calow et al., 2024).

CONCLUSIONS

This study confirms the effectiveness of an integrated system combining EC + OZ for removing COD and TSS from tannery wastewater. Under the best operating conditions, an optimal removal of 92% and 28% was obtained for TSS and COD, respectively. No significant differences were observed in toxicity between the raw and treated samples under the optimal treatment conditions, with the treatment being conducted for 30 min. Considering toxicity as a critical factor for discharging treated effluents into water bodies, the proposed treatment must be supplemented with other technologies that remove recalcitrant compounds and ROS that may have been formed as this ensures the minimization of toxicity in the treated water.

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