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Evaluation of effluent treatment from paiche skin dyeing with juglone using electrocoagulation and ozonation: removal performance and toxicity on *Lactuca sativa*

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ABSTRACT

The application of walnut-based dyes in leather dyeing represents a less polluting process compared to the use of synthetic dyes; however, it still requires the evaluation and treatment of the generated effluents. The present study evaluated the efficiency of COD removal through the application of electrocoagulation and ozonation processes for the treatment of effluents generated during the dyeing of paiche skins using a walnut leaf-based dye. Subsequently, bioassays using Lactuca sativa seeds were conducted to assess the toxicity of both treated and untreated effluents, as well as the natural walnut-based dye and reference synthetic dyes. Optimal conditions for the electrocoagulation process were identified at a treatment time of 30 min and a current intensity of 7 A, achieving a COD removal efficiency of 51.67%. The application of ozonation as a complementary process to electrocoagulation resulted in an additional maximum removal of 12.51%. The bioassays indicated a reduction in toxicity in effluents treated by electrocoagulation compared to the untreated effluent. Regarding the dyes, the natural walnut-based dye exhibited higher toxicity than the synthetic dyes; however, this toxicity is associated with the active compound juglone, which has known phytotoxic activity.

Keywords: electrocoagulation, ozonation, walnut-based dye, phytotoxicity, *Lactuca sativa*.

INTRODUCTION

Wastewater treatment is essential to promote environmental sustainability and mitigate the associated environmental impacts. In the tanning industry, the use of various substances during leather processing generates a considerable amount of effluents containing high concentrations of organic and inorganic substances. According to (Suman et al., 2021), effluents from this industry are classified among the major pollutants within industrial wastewaters. The inadequate discharge of these effluents into the environment causes a significant negative impact (Slama et al., 2021), resulting in irreversible damage to both environmental quality and human health (Saira and Shanthakumar, 2023). Therefore, the proper management and treatment of these effluents are essential to mitigate negative environmental impacts and comply with regulatory requirements.

The tanning industry is structured into three main stages: beamhouse, tanning, and finishing (Barra-Hinojosa and Marrufo-Saldaña, 2020). In the tanning stage, retanning, dyeing, and fatliquoring are intended to improve leather characteristics by providing a more refined texture, greater strength, and color. According to Gómez et al. (2023), the effluent from the dyeing stage contains a large amount of recalcitrant substances; residual dyes, especially synthetic ones, are the main pollutants, since they contain toxic, carcinogenic, and mutagenic chemical elements that affect both aquatic ecosystems and human health. Ardila-Leal et al. (2021) point out that these synthetic dyes are resistant to degradation and have a high capacity for accumulation in organisms. According to Yadav et al. (2023), industries use synthetic dyes because of their high durability, wide range of colors, ease of application, and low cost. However, due to their toxicity, many countries have banned their use, forcing industries to seek substitute natural dyes.

In the past, the tanning industry used natural dyes made from plants, animals, and minerals; however, in the mid-19th century, the discovery of synthetic dyes replaced natural ones. Although synthetic dyes offer a wide range of colors and bright shades, their toxicity has become a problem for both human health and the environment. In contrast, natural dyes are biodegradable, making them a viable alternative for environmental sustainability (Erkan et al., 2014; Prayochmee et al., 2021). For this reason, there has been growing interest in the use of natural dyes by both researchers and industries (Alegbe and Uthman, 2024).

To mitigate the harmful effects of effluents containing synthetic dyes, various treatment techniques have been developed and improved, including physical, chemical, biological, or combined methods. Among these technologies, electrocoagulation (EC) stands out. It is an electrochemical process in which metallic electrodes, generally iron or aluminum, release metallic ions under the action of an electric current (Aguilar-Ascón et al., 2025). These ions become hydrated and generate in situ coagulant species, such as amorphous metallic hydroxides ($Al(OH)_3$, $Fe(OH)_3$) (Tegladza et al., 2021), which act similarly to traditional chemical coagulants, but without the need to add external chemical coagulants (Aryanti et al., 2024; Moussa et al., 2017). These coagulants agglomerate the contaminants present in water and are removed through charge neutralization, adsorption, coprecipitation, and floc formation, which are subsequently separated by sedimentation or flotation, assisted by hydrogen microbubbles produced at the cathode (Garcia-Segura et al., 2017). These flocs are usually larger, more stable, and easier to remove than those generated in conventional chemical coagulation (Aguilar-Ascón et al., 2024). During the electrocoagulation process with aluminum electrodes, anodic dissolution of aluminum occurs, generating Al^{3+} ions, while at the cathode water reduction takes place, producing hydroxyl ions (OH^-) and hydrogen gas (H_2). Kabdaşlı et al. (2012) indicate that electrocoagulation has proven effective for the removal of color, suspended solids, heavy metals, and petroleum-derived products, which are difficult to remove by conventional methods. In recent decades, interest in the use of electrocoagulation to treat effluents containing both organic and inorganic matter has grown considerably (Farhan Abbass et al., 2024).

The efficiency of EC depends on factors such as pH, current intensity, electrolysis time, and effluent composition, which condition both the formation of coagulant species and energy consumption and electrode passivation (Ghernaout et al., 2019). Electrocoagulation offers multiple benefits, such as high efficiency, reduced operating costs, equipment simplicity, ease of process control, lower sludge generation, and lower chemical requirements compared with chemical coagulation techniques (Aryanti et al., 2024; Başaran Dindaş et al., 2020; Solayman et al., 2023).

Regarding dye removal by electrocoagulation, the removal of the synthetic dye Brilliant Green BG, used in tanning, prepared in synthetic water at pH 6.0 and COD concentrations between 660–2065 mg L^{-1}, has been reported using a continuous upflow reactor with parallel plates and 1018 steel as sacrificial electrode, achieving a color removal efficiency of 100% and 86% COD removal (Márquez et al., 2022). Another study on the removal of azo dyes (Acid Red 18) treated with aluminum electrodes showed that color removal efficiency reached 92.3% after 40 minutes and strongly depended on pH (optimum around pH 4) and current density, with a critical point near 26 $mA \cdot cm^{-2}$, where decolorization increased markedly, but further increases significantly raised anode corrosion, sludge production, and energy consumption (Khosravi et al., 2016). Shaker et al. (2020) evaluated the application of electrocoagulation to the acid dye Acid Green 20 (AG20) dissolved in water in a stirred batch system with a bipolar series arrangement, comparing Fe and Al electrodes. Under optimized conditions (pH 2, 40 mA/cm^2, NaCl 0.5 g/L, 9 electrodes), maximum color removal efficiencies of 96.38% (Fe) and 94.81% (Al) were reported.

Electrocoagulation has also been investigated for the removal of phenolic compounds. Kraljić Roković et al. (2014) used steel electrodes, achieving an efficiency greater than 90% in synthetic solutions at pH 5.1. Prayochmee et al. (2021) evaluated treatment in an indigo dyeing effluent with COD concentrations of 2500–3100 mg/L and pH between 10 and 12, using aluminum electrodes, and concluded that removal is greater when the initial treatment pH is below 7, since at high pH the efficiency decreases due to lower formation and stability of aluminum hydroxide. The achieved COD removal efficiency was 73%.

On the other hand, ozonation is an advanced oxidation process (AOP) that uses ozone (O_3), which has a high oxidizing power capable of mineralizing organic compounds and degrading color-causing compounds that are resistant to biological treatments (Beltran, 2003). In the aqueous medium, ozone can decompose to generate hydroxyl radicals (•OH), which have a high oxidation potential and promote the oxidation of various compounds present in water.

Ozonation is also considered an innovative technology for effluent treatment because it can reduce operating costs and reaction times while achieving high efficiencies in the degradation of chemical substances present in effluents (Malik et al., 2020). This technique is effective in significantly improving biodegradability and eliminating refractory organic compounds. These refractory contaminants are characterized by high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and they are resistant to biological treatments due to their low biodegradability index (Das et al., 2024). Likewise, ozone is an excellent disinfectant and can be used to inactivate microorganisms such as protozoa, which are highly resistant to conventional disinfectants (von Gunten, 2003). In the case of dyeing and tanning effluents, ozonation has been shown to significantly reduce dyes and COD, in addition to improving wastewater biodegradability (Preethi et al., 2009).

Preethi et al. (2009) evaluated ozonation treatment in a synthetic sample prepared with the azo dye Direct Brown (C.I. 26230) and in a real tannery effluent with approximately 5000 mg/L COD, determining that removal efficiency decreases from 60 to 20% as COD concentration increases from 2000 mg/L to 5000 mg/L for an ozonation time of 80 minutes. Likewise, it was concluded that increasing pH improves COD removal due to greater hydroxyl radical formation; in contrast, decolorization may be favored at lower pH by the selective attack of molecular ozone on chromophore groups.

Kasiri et al. (2013) studied the decolorization of solutions of three acid azo dyes used in leather dyeing: Acid Black 1, Acid Yellow 19, and Acid Orange 7. The treatment was carried out in a 500 mL batch reactor equipped with a magnetic stirrer and thermometer, supplied with ozone continuously generated at 600 mg/L, resulting in color removal between 80 and 93%. For its part, Lanzetta et al. (2023) applied ozonation to a tannery effluent using a system with an ozone generator, a 750 mL glass contact column (filled with 0.5 L), porous stone injection, air supplied at 4.5 $L \cdot min^{-1}$, and an ozone rate of 12.5 $mg \cdot h^{-1}$; at an initial pH of 8.2 and pH adjusted to 10, with contact times of 15, 30, and 45 min, determining that at 45 min color removal was low (21% without pH adjustment and 26% at pH 10) and COD removal remained around 33% at pH 10.

Regarding the removal of natural organic compounds by ozonation, an efficiency of 27% has been reported for the removal of vegetable tannins (polyphenolic compounds) from effluents with COD concentrations of approximately 25,000 mg/L, where 72–76% was estimated to originate from tannins (Balakrishnan et al., 2020). In turn, Kalyanaraman et al. (2015) used ozonation in synthetic solution of wattle extract to remove a COD of 1150 mg/L, achieving an efficiency of 20%.

The tanning of paiche skins is an activity that adds value to the skin. This activity, which also constitutes a circular economy practice, is a link in the manufacture of high-value apparel products for niche markets, generating a productive chain with economic and social impact in the places of origin (Segundo Espada et al., 2020). Currently, there is growing demand for sustainable materials to be used in the fashion industry, and fish leather fits well within this concept. Thus, its visibility in international fashion increased during the 2022–2024 period (media and brands), suggesting a growing projection in demand for this skin (New York Post, 2022). However, the artisanal or industrial production of this type of leather requires a review of the production process for the application of clean technologies that minimize the environmental aspects generated and that put environmental health at risk. In this sense, the substitution of synthetic dyes with natural ones is a feasible alternative that further increases the product's value because it can incorporate products from biodiversity, by-products from other value chains, and, in some cases, ancestral knowledge.

In this research, walnut leaves have been used as a source of the dyeing principle 5-hydroxy-1,4-naphthoquinone (juglone). This dye is traditionally used in the dyeing of textile fibers such as cotton, silk, linen, and nylon, providing natural brown shades (Marrufo-Saldaña et al., 2024; Ojstršek and Fakin, 2019). Walnut is a species with dual economic use, both as fruit and as timber. In Peru, the main walnut species is Andean walnut (*Juglans neotropica*). In 2023, SERFOR reported the production of 5,204.40 m^3 of roundwood and 6,166.13 m^3 of sawn wood. Considering a wood density of 0.52 t/m^3 (Toro Vanegas and Roldan Rojas, 2018), a biomass expansion factor (BEF) between 1.3–1.6 for mature forests and a leaf mass fraction (LMF) in mature trees of approximately 1–3% of aboveground biomass, for broad leaves such as walnut leaves (Nguyen et al., 2012), availability is estimated between 35 and 164 tons of leaves, which can be valorized as a source of natural dyes (Postigo-Sobrevilla et al., 2023).

The tanning of paiche skins and their dyeing with natural dyes represent a more environmentally friendly alternative compared to the use of synthetic dyes, contributing to the growing demand for sustainable materials. However, this process still generates effluents whose quality and environmental impact must be properly assessed. In this context, both physicochemical characterization and toxicity evaluation are essential to ensure an adequate assessment of these effluents. Nevertheless, studies addressing electrocoagulation treatment and toxicity evaluation in real effluents from leather dyeing processes remain limited.

The present study aims to evaluate the efficiency of electrocoagulation and ozonation processes for the removal of contaminants in wastewater generated from the dyeing stage of the tanning industry. To this end, COD was characterized as the main indicator of pollutant load; electrocoagulation and ozonation treatments were applied to effluents from the dyeing of paiche skins using a natural dye extracted from walnut leaves; and the toxicity of treated and untreated effluents was assessed through bioassays using lettuce seeds (*Lactuca sativa*) as a bioindicator organism. In this way, the study contributes to the environmental assessment of tanning processes, supporting the development of technologies that consider and mitigate their associated environmental impacts.

MATERIALS AND METHODS

Raw materials and reactants

Paiche skins were obtained from the Pucallpa region in Peru and originated from local aquaculture activities. Walnut leaves were purchased from a local market in the city of Lima. The synthetic dyes Beige Ambranile PG and Pardo Ambranile GS from Codyeco brand were applied to compare toxicity with walnut-based dye, and fatliquoring agent (Fospholicker 6146-L) was applied in the dyeing process. For the toxicity assays, seeds of *Lactuca sativa* (long light-green romaine lettuce) from the BATTLE brand, lot 002/NI 022, were used. Reagents utilized included calcium sulfate dihydrate (precipitated for analysis, EMSURE®), zinc sulfate heptahydrate (ACS, ISO, Reag. Ph Eur, EMSURE®), potassium chloride (reagent grade, Scharlau®), sodium bicarbonate (reagent grade, ACS, ISO, Scharlau®), and anhydrous magnesium sulfate (extra pure, Scharlau®).

Skin dyeing

The dyeing process was carried out at the CITEccal Lima tannery pilot plant. Paiche leather tanned with glutaraldehyde and phenolic compounds (Segundo Espada et al., 2020) was dyed with a natural dye solution obtained from walnut leaves (Marrufo-Saldaña et al., 2024) at a concentration of 200% relative to the wet weight of the skins. Simultaneously, 10% salt was added to promote dye fixation within the collagen structure.

The skins were then placed in a tanning drum, where they remained under agitation for 90 min, allowing dye absorption. Subsequently, the fatliquoring agent was added at a proportion of 2% to improve the flexibility and mechanical resistance of the material and to prevent excessive stiffness after drying. After an additional 60 min of rotation, a dye penetration test was performed by making a cross-sectional cut in the skin to evaluate color uniformity and fixation. Finally, the dyed skins were removed and dried, obtaining a product with the characteristic walnut shade, ready to proceed to the finishing stage. As a result of the process, an effluent containing dye residues, salts, and other compounds derived from products used in the tanning process was generated. The dyeing process scheme is presented in Figure 1.

Effluent sampling and characterization

Effluent sampling was carried out at the end of the dyeing process, and the obtained effluents were characterized to evaluate COD using the SMEWW-APHA-AWWA-WEF-Part 5220 D method, based on closed reflux and colorimetric measurement. Effluent characterization was performed in a laboratory accredited within the scope of the method. In addition, effluent samples were stored for the treatment tests, in which 1.7 L was used for each electrocoagulation treatment and 0.5 L for the ozonation treatment.

Effluent treatment by electrocoagulation

A batch-type reactor was used to carry out effluent treatment by electrocoagulation. The reactor dimensions were 14 × 10 × 17 cm, with an approximate capacity of 2.4 L. The electrocoagulation system consisted of eight aluminum electrodes arranged in series, of which four acted as anodes and four as cathodes. Each electrode measured 7 × 7 cm, with a surface area of 49 cm². The interelectrode spacing was 1 mm, which favored process efficiency by optimizing contact between the effluent contaminants and the coagulant species generated in situ.

A 3^2 factorial design was used, in which the studied variables were current intensity (x1) and treatment time (x2). The levels applied for current intensity were 3, 5, and 7 A, and for treatment time were 10, 20, and 30 min. The experimental design for the electrocoagulation treatment is shown below. This experimental stage was carried out at the Scientific Research Institute of the Universidad de Lima.

Effluent treatment by ozone

Ozone treatment was applied to the effluent previously treated by electrocoagulation (optimal COD removal condition). Ozonation was carried out at a nominal ozone generation rate of 1 g/h for 30, 60, 90, and 120 min using a DADAWYNDY ozone generator, model AZ-IG-G (maximum production 1000 mg/h, nominal power 10 W), supplied with ambient air by an air pump. The treatment volume per run was 500 mL. The ozonized gas was introduced into the reactor through a hose connected to a porous stone diffuser located at the bottom of the tank, generating fine bubbles to promote gas dispersion and mass transfer into the effluent. Under these conditions, the dosing rate remained constant, and the accumulated ozone dose depended on the

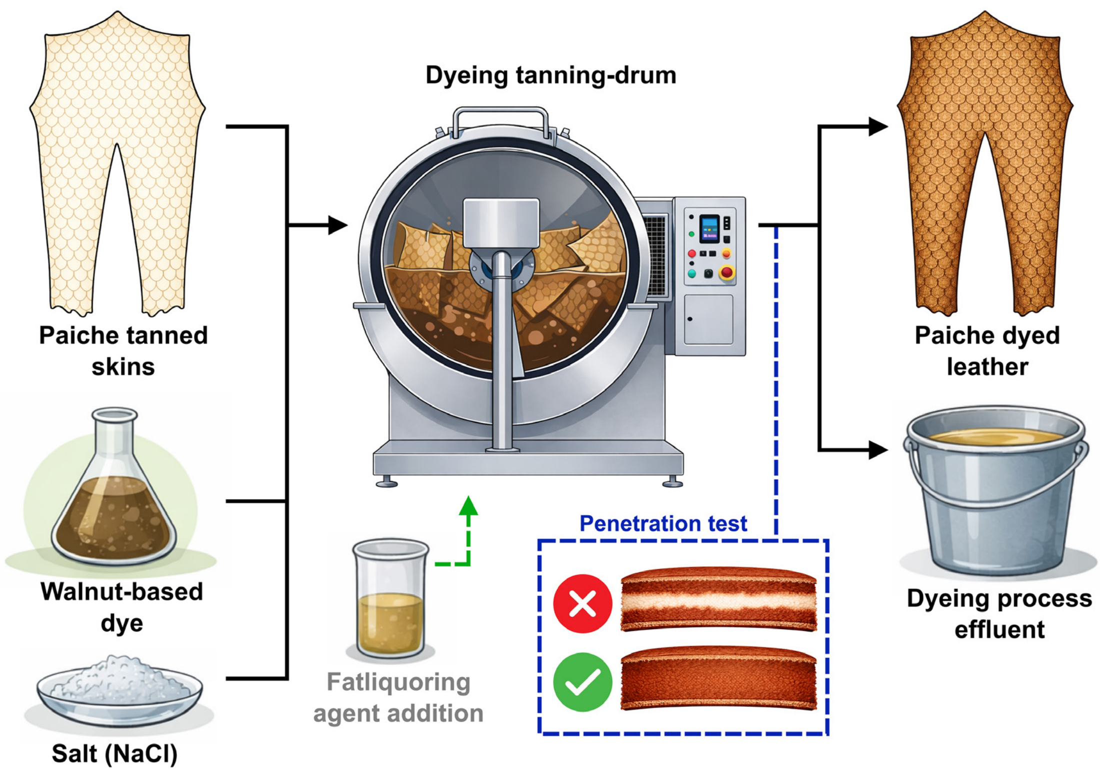

Figure 1. Dyeing process using a walnut-based dye

contact time. A schematic representation of the overall treatment process is shown in Figure 2.

UV spectrophotometric analysis

Samples of the raw effluent and electrocoagulation-treated effluents were analyzed by UV-visible spectrophotometry. These spectra were compared with that obtained for a walnut dye sample, from which the characteristic spectrum of the active compounds composing the extract was obtained, in order to evaluate dye removal by electrocoagulation. An Agilent Cary 60 UV-Vis spectrophotometer with Cary WinUV software was used. These measurements were performed at the Chemistry Laboratory of CITEccal Lima.

Toxicity assays

The toxicity of raw and electrocoagulation-treated effluent samples was evaluated by a phytotoxicity assay using *Lactuca sativa* seeds. This species is widely used because of its sensitivity and rapid germination. This plant bioindicator is among the species recommended by the United States Environmental Protection Agency (US EPA, 1996) for ecotoxicological studies (Aguilar-Ascón et al., 2024).

The assay included four experimental groups: negative control (reconstituted water), positive control ($ZnSO_4$), untreated effluent, and effluent treated by electrocoagulation. The negative control was prepared by adapting the methodology established in the Mexican standard NMX-AA-087-1995-SCFI. In a volume of 2 L, 0.2400 g of $MgSO_4$, 0.3840 g of $NaHCO_3$, 0.0160 g of KCl, and 0.2400 g of $CaSO_4$ were dissolved in distilled water. For the positive control, a stock solution of 500 mg/L $ZnSO_4$ was prepared in a 200 mL volumetric flask. From this solution, $ZnSO_4$ concentrations of 10%, 20%, 30%, 40%, and 50% were prepared and diluted with reconstituted water. Effluent samples (raw and treated) were diluted with reconstituted water at concentrations of 1%, 3%, 10%, 30%, and 100% in order to evaluate the dose-response effect.

For each treatment, 20 *L. sativa* seeds were placed in 90 mm Petri dishes using 3 mL of sample per dish. Three independent replicates were performed for each concentration. The seeds were incubated for five days at 22.2 ± 0.5 °C in darkness.

At the end of the incubation period, the root length of the germinated seedlings was measured using graph paper. This parameter was used as a quantitative indicator of the toxic effect in comparison with the negative control.

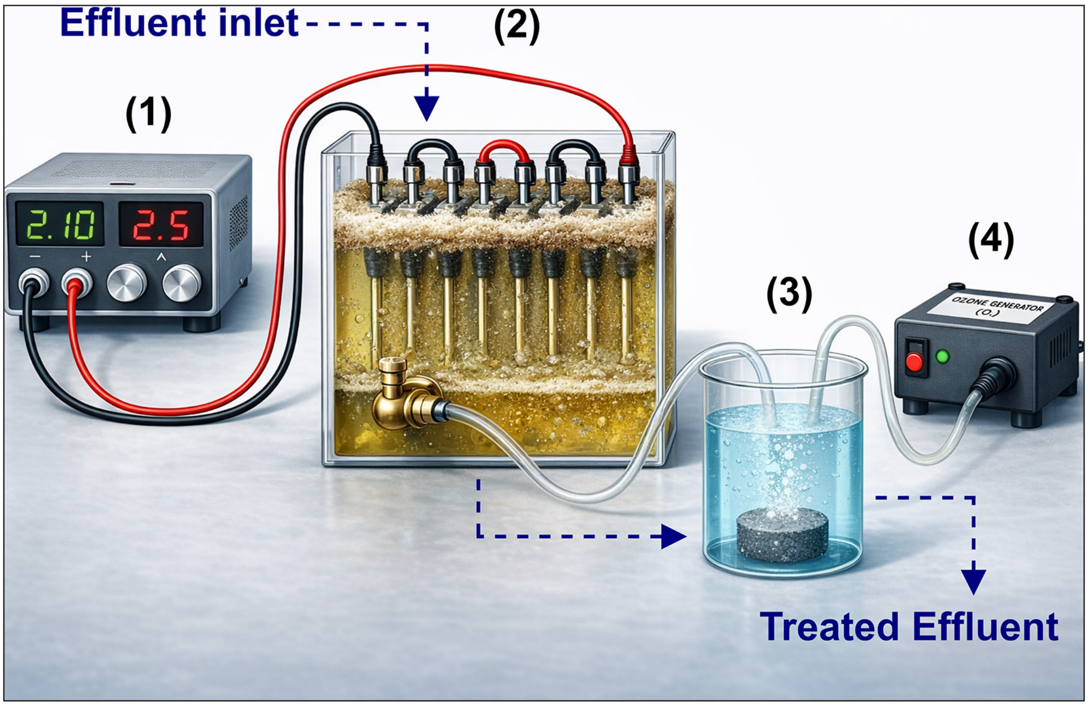

Figure 2. Electrocoagulation and ozonation process diagram: (1) power supply, (2) electrocoagulation reactor, (3) ozonation recipient, (4) ozone generator

To estimate the inhibition coefficient (EC_{50}), a linear regression model was applied from the dose-response curve, allowing the determination of the concentration at which 50% of germination in *Lactuca sativa* seeds was inhibited. Once the EC_{50} value was calculated, toxicity units (TU) were estimated according to Equation 1) which were used as a criterion to classify the acute toxicity level of the analyzed sample. Table 1 presents the toxicity classification according to the toxicity unit value (Aguilar-Ascón et al., 2024).

$$TU = \frac{1}{EC_{50}} \times 100 \quad (1)$$

Absolute germination (AG) and germination index (GI) were also evaluated. These indicators allow the comparison of sample toxicity at the different concentrations tested. The calculation of these indicators was carried out according to the following equations.

$$AG = \frac{N_{germ}}{N_{seed}} \quad (2)$$

$$GI = \frac{N_{germ}}{N_{cont}} \times \frac{RL_{germ}}{RL_{cont}} \quad (3)$$

where: N_{seed} is the number of seeds sown, N_{germ} is the number of germinated seeds, Ncont is the number of seeds germinated in the negative control, RL_{germ} is the average root length of lettuce seedlings in the sample, and RL_{cont} is the average root length of lettuce seedlings in the negative control.

GI values above 1.2 indicate a growth stimulation effect (A); values between 0.8 and 1.2 indicate no significant growth effect (B); values between 0.4 and 0.8 indicate slight growth inhibition (C); values lower than 0.4 indicate significant growth inhibition (D); and a value of 0 indicates total inhibition of growth and germination (E). The toxicity assay was carried out at the R&D Laboratory of CITEccal Lima.

Data processing and statistical analysis

Statistical analysis was carried out using RStudio software version 4.5.2 (R Core Team, 2025). The experimental results of the factorial design were evaluated using the response surface methodology (RSM) in order to determine the optimal process conditions. For this purpose, different fitting models (linear, quadratic, and mixed) were analyzed to identify the one that best represented the behavior of the data.

For the analysis of the factorial design, the packages "rsm", "MASS", "lmtest", and "FrF2" were used, allowing model fitting, evaluation of factor significance through ANOVA, and assessment of model assumptions. The response surface and contour plots obtained facilitated visualization of the combined effect of the factors on the response variable.

The analysis of toxicity assay results was performed using the packages "readr", "plyr", "dplyr", "tydir", "tydiverse", "ggplot2", "car", "FSA" and "PMCMRplus". The results were evaluated using non-parametric tests, applying Kruskal-Wallis analysis for comparisons among samples and the Conover-Iman test with Holm adjustment for multiple comparisons. The analysis was performed to compare groups and the evaluated sample concentrations, and finally, least-squares linear regression was applied for the determination of EC_{50}.

Table 1. Toxicity units classification

TU	Class	Toxicity
TU < 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

RESULTS AND DISCUSSION

Effluent characterization

Characterization of the dyeing effluent containing walnut leaf extract showed a COD of 7,870.0 mg/L and a BOD of 1407.5 mg/L, greatly exceeding the maximum limit of 1,000 mg/L established by Supreme Decree No. 010-2019-VIVIENDA (DS 010-2019-VIVIENDA, 2019). Thus, the BOD/COD ratio was 0.18, suggesting low biodegradability and the possible presence of recalcitrant organic compounds.

The effluent pH ranged between 4 and 5, an acidity attributable to the use of organic acids during the dyeing process and to the acidic nature of the tannins present in walnut leaves. Consistent with this, Masías Brocker (2007) reported an approximate pH of 5.71 for walnut leaves.

Likewise, a recent study developed by Ojstršek et al. (2022) reported a concentration higher than 7200 mg/L for wastewaters from the natural dyeing of wool fibers with common walnut leaves extract and a pH of 4.2.

No specific reports were found in the scientific literature on COD in leather dyeing effluents using natural dyes. Table 2 compiles COD values ranging from 6,816.74 to 41,689 mg/L in tannery dyeing effluents and from 1179 to 4000 mg/L in textile effluents, also dyed with synthetic dyes.

The high COD concentrations in leather dyeing effluents are associated with the presence of dyes, surfactants, retanning agents, and especially fatliquors; reviews have reported that these compounds contribute to an effluent with high COD and poor biodegradability, and that fatliquoring agents may account for the highest COD load (Hansen et al., 2021).

The final condition of the dyeing effluent in the present study, with a pH between 4 and 5, is ideal for electrocoagulation, since when aluminum electrodes are used in a near acid-to-neutral range, the formation of polymeric hydroxides and eventually $Al(OH)_3(s)$ is favored, which in turn promotes sweep coagulation and adsorption, whereas at high pH, $Al(OH)_4^-$ (soluble) is formed and floc generation/effectiveness decreases (Khosravi et al., 2016; Márquez et al., 2022). Shaker et al. (2020), report a pH range between 2 and 6 for the removal of synthetic dyes by electrocoagulation.

Effluent treatment by electrocoagulation

Table 3 shows the COD removal results obtained in the experimental design applied to paiche leather dyeing effluent with walnut leaf extract. The analysis of the experimental design was performed using ANOVA, fitting the results to a second-order model with linear and quadratic components for both variables. The results are presented in Table 4. The model was found to meet the assumptions of normality, homoscedasticity, and independence of errors. The model showed a high degree of fit, with a coefficient of determination R^2 of 97.62% and an adjusted R^2 of 95.25%. The model equation is presented below, and the response surface plot is shown in Figure 3. Significant linear effects from both variables were identified and the most efficient treatment for COD removal was achieved at 7 A for 30 min, reaching 51.67% COD removal. The removal percentage was estimated relative to the initial COD concentration of the effluent.

To evaluate the effect of current independently, treatments were compared at constant time. At 10 min, removal increased from 15.84% (3 A) to 18.43% (5 A) and 29.80% (7 A), showing that an increase in current intensity, and therefore current density, improves removal during the initial stages. At 20 min, the 3 and 5 A treatments showed very similar removals (24.48% and 25.04%), whereas increasing to 7 A markedly enhanced removal (40.78%), indicating that the effect of

Table 2. Comparative COD concentrations in dyeing effluents containing natural or synthetic dyes

Matrix effluents	COD concentration (mg/L)	Colorant type	Dyeing principle	References
Leather	41,689.00	Real conventional process	-	Khelali et al. (2025)
Leather	6,816.74 – 9,887.68	Synthetic	Acid red 357, Acid Blue 161 and Acid Black 210	Ortiz Monsalve (2017)
Leather	38,169.00	Synthetic	-	Saha et al. (2025)
Leather	7,755.00	Synthetic	-	Venkataraman et al. (2022)
Textile	3,042.00	Synthetic	Indigo	Rendón et al. (2023)
Textile and wood	> 3,000.00	Real effluent	Walnut Leaves	Ojstrek et al. (2022)
Textile	4,000.00	Synthetic	-	Venkataraman et al. (2022)
Textile	1,314.00	Synthetic	-	Carrasquero Ferrer et al. (2024)
Textile	720.00	Synthetic	-	Zafar et al. (2024)
Textile	404.00	Synthetic	-	Gasmi et al. (2022)
Textile	400.00	Synthetic	-	Jiménez -Pacheco et al. (2022)

Notes: “Matrix effluents” corresponds to the origin of the effluent. “Colorant Type” denotes whether the effluent corresponds to a real or synthetic sample

Table 3. COD removal results. “Treatment” denotes the code assigned to each experimental run

Treatment	x1: Current intensity (A)	x2: Treatment time (min)	DQO (mg/L)	DQO removal (%)
EC310	3	10	6623.00	15.84
EC320	3	20	5943.60	24.48
EC330	3	30	4298.90	45.38
EC510	5	10	6419.80	18.43
EC520	5	20	5899.10	25.04
EC530	5	30	4406.90	44.00
EC710	7	10	5524.50	29.80
EC720	7	20	4660.90	40.78
EC730	7	30	3803.60	51.67

Table 4. ANOVA for the experimental design results

Parameter	Estimate	Std.Error	t-value	Pr(>|t|)
(Intercept)	0.264324	0.020994	12.5903	0.0002291
x1	0.060917	0.011499	5.2975	0.0060973
x2	0.128291	0.011499	11.1566	0.0003674
x1^2	0.055	0.019917	2.7615	0.0507735
x2^2	0.040883	0.019917	2.0527	0.1093579

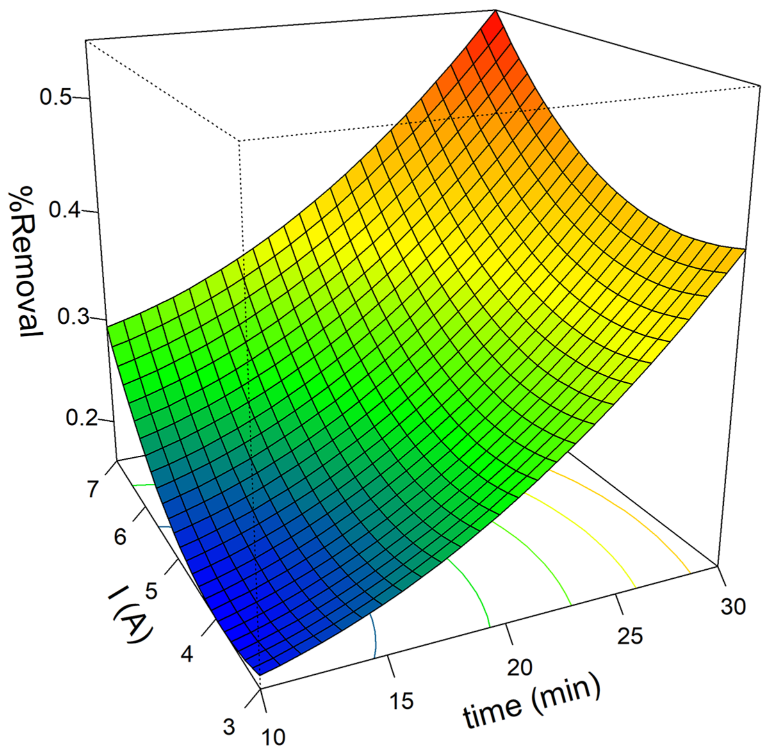

Figure 3. Response surface graph for COD removal

current becomes more evident mainly at higher intensities. At 30 min, the effect of increasing current became attenuated, since removal was similar between 3 and 5 A (45.38% and 44.00%) and increased only moderately at 7 A (51.67%).

This behavior agrees with findings reported in the literature, which indicate that increasing current density enhances process efficiency due to greater anodic dissolution and the in-situ generation of metallic hydroxides and oxyhydroxides that retain contaminants through adsorption and sweep flocculation, as well as the increase in gaseous bubbles (H_2/O_2) that promote floc formation and separation. However, above certain thresholds, diminishing returns may be observed together with higher energy requirements and greater sludge production (Khosravi et al., 2016; Nandi and Patel, 2013).

Likewise, when analyzing the effect of time at constant current, a sustained increase in removal is observed between 10 and 30 min, consistent with the fact that longer treatment time implies a greater applied charge (I·t) and greater production of coagulant species. However, the increase in removal tends to diminish at longer times, suggesting diminishing returns and that a fraction of the remaining organic matter may be predominantly dissolved or less susceptible to coagulation within the evaluated range. These results are consistent with reports describing an initial rapid phase, commonly within 10–30 min, followed by marginal improvements as adsorption/entrapment equilibrium in flocs is approached (Jing et al., 2020). In the present study, this behavior is observed as increasing current density and treatment time enhanced COD removal, although a plateau trend was observed, which could be attributed to the presence of a less coagulable organic fraction under the studied conditions.

To compare these results with the compilation of studies in Table 5, it is essential to distinguish between synthetic samples and real effluents, and to consider both the initial COD and the applied load. In synthetic samples, where dyes are dissolved in water, high efficiencies are reported because the matrix is simple and COD is dominated by the dye itself; for example, for Brilliant Green, 96% COD removal and 100% color removal have been reported. In contrast, in real effluents, COD includes mixtures of dissolved organic compounds and auxiliaries, which tends to limit the achievable percentage with EC compared with synthetic systems.

Even so, real effluents with moderate initial COD may exhibit high removal when optimized conditions are applied; for example, in a real indigo effluent (2500–3100 mg/L), 73.13% COD removal at 30 mA/cm^2 for 60 min has been reported, which implies a higher electrochemical load and a lower initial COD concentration than in the present study, favoring higher removal (Prayochmee et al., 2021). Overall, the maximum COD removal achieved in this study (51.67%) is within the range reported for real high-polluted wastewaters in previous studies.

It should be noted that textile and tannery effluents are not equivalent in terms of pollutant load and composition. In tannery effluents, according to Table 2, very high loads have been reported; for example, a real tannery dyeing effluent presents a COD of 38,169 mg/L. In contrast, textile studies commonly work with lower loads; for example, the real indigo effluent used for electrocoagulation optimization presented a COD of 2.500–3.100 mg/L. Nevertheless, even within textile effluents, natural dyeing may generate high organic loads: effluents from wool dyeing with walnut leaf extract have been reported to reach COD values up to 7200 mg/L, suggesting a significant contribution from polyphenols, tannins, and other extracted organic compounds (Ojstršek et al., 2022).

However, the difference between textile and tannery effluents lies not only in COD concentration but also in the nature of the chemical substances present. Tannery effluents include complex mixtures of surfactants, oils, resins, tannins, among other compounds, which make removal more difficult. In tannery dyeing effluents, high decolorization may occur together with limited COD removal due to the high fraction of dissolved organic matter associated with these auxiliary chemicals (Aygun et al., 2021).

It has been reported that COD removal by electrocoagulation strongly depends on compound solubility: when the organic load is predominantly in the dissolved phase, COD decreases to a lesser extent (Ardhan et al., 2022). In the case of dyeing with walnut leaves, the solubility of the dyeing principle juglone has been reported to be around 52 mg/L (Strugstad and Despotovski, 2013), which is not considered high; however, extraction of the plant material also incorporates phenolic acids and tannins, several of which show greater affinity for the aqueous phase, contributing to the dissolved organic matter load (Santos et al., 2013; Sójka et al., 2019). Therefore, part of the COD may remain as a dissolved fraction, limiting removal by electrocoagulation, and in the case of tannins, additionally contribute to a more slowly degradable COD fraction; indeed, tannin extracts such as mimosa/chestnut have been reported to generate COD of a recalcitrant and persistent nature (Toscanesi et al., 2022).

On the other hand, it is important to emphasize that many studies are conducted with synthetic

Table 5. Electrocoagulation effectiveness in COD and color removal from dyeing effluents. "Electrodes" indicates the type or material that electrodes were made of "pH", "Current Intensity/Voltage", and "Time (min)" denotes the experimental conditions for electrocoagulation

Matrix effluents	Initial COD (mg/L)	Electrodes	pH	Current Intensity / Voltage	Time (min)	Removal (%)	References
Textile dyeing effluent	720.00	Al-Al	7.0	15 V	60	79% COD 86% Color	Zafar et al. (2024)
Textile dyeing effluent	404.00	Al-Al	9.0	4 V	36.26	63.05% COD 99.07% Color	Gasmi et al. (2022)
Textile dyeing effluent	-	Al-Al	5.02	40.00 mA cm^{-2}	49.99	93.58% COD	Jiménez Pacheco et al. (2022)
		Fe-Fe	9.62	40.00 mA/cm^2	39.75	87.08% COD	
Tanning Brillant Green colorant - synthetic	660.00 2,065.00	Steel	6.0	6 mA/cm^2	-	96% COD 100% Color	Márquez et al. (2022)
Tanning Acid Red 18 colorant – synthetic	-	Al	4.0	26 mA/cm^2	40	92.3% Color	Khosravi et al. (2016)
Tanning Acid Green 20 (60 mg/L) colorant - synthetic	-	Fe	2.0	40 mA/cm^2	30	96.38% Color	Shaker et al. (2020)
		Al			20	94.81% Color	
Phenolic compounds - synthetic	-	Steel	5.1	10 mA/cm^2	35	96.7% phenols	Kraljić Roković et al. (2014)
Indigo dyeing effluent - synthetic	2,500.00 - 3,100.00	Al	<7	30.0 mA/cm^2	60	73.13% COD 94.68% Color	Prayochmee et al. (2021)
Blend of methyl orange (184 mg/L), congo red (130 mg/L) and acid blue-113 (96 mg/L - synthetic	-	Steel	5.3	10.0 mA/cm^2	30	99% Color 81.9% COD	Sugha et al. (2025)
Blend of Acid Blue 113, Acid Blue 29, and Brilliant Green - synthetic	450.00	-	-	-	-	32–48%	Sugha et al. (2025)
Congo red (hasta 100 mg/L) - synthetic	-	Al	8	200 mA/cm^2	60	93% Color	Sankar et al. (2018)
Post-tanning real effluent, including dyeing process (dark-red)	-	Carbon-Steel	3	6.4 mA/cm^2	-	23% COD	Villaseñor Basulto et al. (2022)

samples, in which only the dye, salt, or some mordant is usually present, so the coagulant generated by electrocoagulation acts on a simpler load. In real effluents, such as the one studied in the present investigation, there may be competition for the coagulant due to surfactants, oils, resins, tannins, and salts present in the effluent, making it more likely that COD removal stabilizes at moderate values.

UV spectrophotometric analysis

The UV spectrophotometric analysis withdrawn in Figure 4, shows an absorbance curve in the 200–400 nm region. The walnut dye extract shows an intense UV band with a maximum around 210 nm, consistent with what has been reported for juglone (5-hydroxy-1,4-naphthoquinone) in aqueous extracts (Marrufo-Saldaña et al., 2024).

At constant time (60 min), increasing the current from 2 A (EC260) to 4 A (EC460) and 6 A (EC660) systematically reduced absorbance relative to the raw effluent (MI-Effluent > EC260 > EC460 > EC660), suggesting the removal of juglone and, more generally, other phenolic and aromatic fractions, including walnut polyphenols (Lama-Muñoz and Contreras, 2022). This decrease is consistent with adsorption and entrapment mechanisms in aluminum hydroxide flocs. In addition, the trend agrees with reports showing that increasing current density enhances coagulant production through anodic dissolution and bubble generation, thereby favoring contaminant removal, including COD, although with diminishing returns at excessively high current densities (Kermet-Said and Moulai-Mostefa, 2015).

Ozonation treatment results

The ozonation process results obtained for sample EC660 show an additional removal of chemical oxygen demand (COD) under the evaluated conditions (pH close to 5 and 1 g O_3/h) are presented in

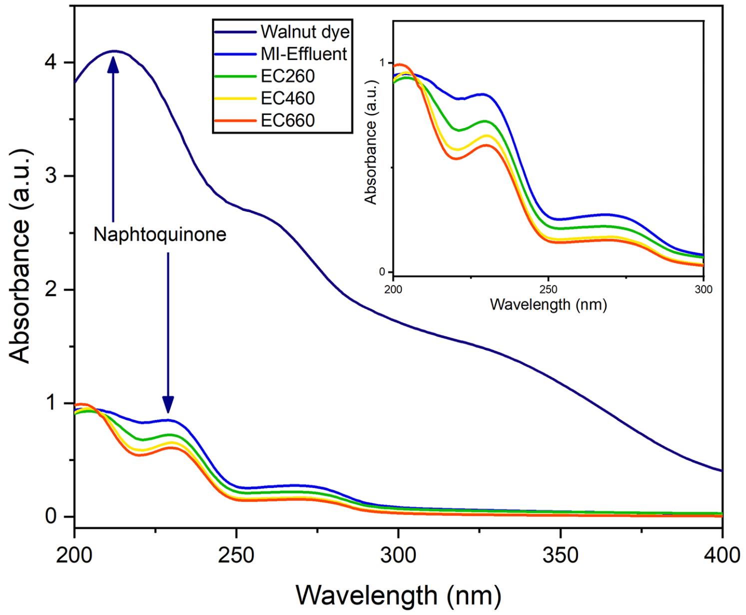

Figure 4. Absorption spectrum of walnut dye and initial and treated effluents

Table 6. The initial COD was 4072.7 mg/L, and the highest removal efficiency was reached at 120 min, with 12.51%. COD removal did not show a strictly monotonic trend, for example a lower value was observed at 90 min, which may be attributed to experimental variability in the COD assay and/or to matrix complexity; therefore, the results are interpreted in terms of the overall process performance and the maximum observed at 120 min.

Overall, these results suggest that ozone contact time influences efficiency in a variable manner, probably due to the nature of the organic compounds present and to limitations associated with oxidant transfer and consumption. In ozonation, reactivity strongly depends on the type of compounds: structures with aromatic rings and unsaturations are more susceptible to attack, whereas more saturated aliphatic fractions and certain oxygenated compounds may persist as partial oxidation products, which may result in moderate COD reductions even when chemical transformation occurs (Kalyanaraman et al., 2015). Likewise, it has been documented that ozonation can substantially contribute to decolorization, whereas mineralization, and therefore total COD reduction, may be limited, especially in complex matrices, which explains why COD removal levels are relatively low in some real effluents (Lanzetta et al., 2023).

In the particular case of this research, the effluent not only contains compounds derived from walnut dyeing, but may also incorporate substances from the tanning process itself; in this study, phenolic syntans and glutaraldehyde were used, making the presence of residual carbonyl compounds plausible. Under ozonation, moreover, the formation of oxygenated intermediates such as aldehydes, ketones, and carboxylic acids from the oxidation of dissolved organic matter, including aromatic/polyphenolic fractions, is common, and these compounds may maintain an appreciable oxygen demand in the COD assay (Lim et al., 2022).

Comparison with previous studies presented in Table 7, supports the variability of the results as a function of matrix type and the species present. For example, in a real final effluent from a tannery treatment plant, with an initial COD of 457 mg/L, COD removal on the order of 33% at pH 10 after 45 min has been reported, whereas color removal remained low, 21% without pH adjustment at pH 8.2 and 26% at pH 10 (Lanzetta et al., 2023).

On the other hand, the importance of distinguishing treatment in synthetic samples versus real effluents, and of considering pH and the "effective" ozone dose, is also evident. For example, in the study by Preethi et al. (2009), high removals were reported in a synthetic azo dye matrix, Direct Brown, 300–500 mg/L, reaching 92% COD removal and 100% color removal at pH 11, flow 6×10^{-3} m^3/min, and 40–60 min, whereas for a real tannery effluent, with an initial COD of 5000 mg/L, under the same pH and flow conditions, COD removal was much lower, 20%, even at a longer treatment time.

Table 6. Effluents treated by electrocoagulation and ozonation

Treatment	DQO (mg/L)	DQO removal (%)
EC660	4072.7	-
TD-OZ-30	3677.4	9.71
TD-OZ-60	3721.9	5.61
TD-OZ-90	3893.3	4.40
TD-OZ-120	3563.1	12.51

In synthetic solutions, in contrast, higher decolorization and/or COD efficiencies are observed; for example, for Acid Black 52 in a packed-bed reactor, 59.8% COD removal at pH 4.8, 4 g/h, and 17.1 min has been reported, which is consistent with a simpler and more controlled matrix (Vedaraman et al., 2013).

It should be noted that ozone decomposition is strongly pH-dependent: at high pH, the formation of hydroxyl radicals (OH^-) from ozone decomposition is favored, whereas at low pH, molecular ozone predominates as the oxidant. Decolorization is usually favored by the direct attack of molecular ozone, which is selective toward chromophores, at more acidic pH, whereas radical oxidation, which is less selective and has a higher oxidation potential, tends to predominate under alkaline conditions, in some cases favoring greater COD reductions (Lanzetta et al., 2023; Preethi et al., 2009).

In summary, after electrocoagulation, the fraction of COD removable by coagulation is significantly reduced, leaving an effluent with a higher proportion of dissolved and potentially more refractory organic matter. Under these conditions, including a pH around 5, ozonation would tend to behave as a partial oxidation process, degrading chromophores and aromatic structures associated with natural dyes and other compounds, but generating oxygenated intermediates that still maintain an appreciable oxygen demand in the COD assay. This could explain why, despite the contact time, the net decrease in COD remains at moderate values. In this sense, a more detailed analysis of by-products, pH effects, and transformation pathways could guide the design of complementary treatments, for example O_3/H_2O_2, catalysis, or integration with a biological stage, especially when the objective is to reduce COD to the levels required by environmental regulations.

Toxicity assays

The results of the acute toxicity assay using *Lactuca sativa* seeds are presented in Tables 8 and 9. The first table shows the toxicity results of the walnut-based dye and two synthetic dyes with similar shades, while the second presents the results for effluents generated after dyeing and those treated by electrocoagulation. Additionally,

Table 7. Ozonation effectiveness in COD and color removal from dyeing effluents; "Ozone flow rate", "Time (min)", and "pH" denotes the experimental conditions for ozonation process

Matrix effluents	Initial COD (mg/L)	Ozone flow rate	Time (min)	pH	Removal (%)	References
Azo Direct Brown dye (300-500 mg/L) - synthetic	-	6 × 10^{-3} m^3/min	40–60	11	92% COD 100% Color	Preethi et al. (2009)
Tanning effluent	5,000.00	6 × 10^{-3} m^3/min	80	11	20% COD	Preethi et al. (2009)
Blend of Acid Black 1 (13 mg/L), Acid Yellow 19 (15 mg/L) and Acid Orange 7 (14 mg/L) - synthetic	-	560–740 mg/L	5	6.2–7.6	80–93% Color	Kasiri et al. (2013)
Tannery effluent treatment plant	457.00	12.5 mg/h	45	8.2	21% Color	Lanzetta et al. (2023)
				10	26% Color 33% COD	
Vegetable tanning with tannins effluent	25,450.00	3 g/h	30	3.5	27% Tannins (improvement of BOD/COD ratio)	Balakrishnan et al. (2020)
Synthetic solution of wattle extract	1,150.00	2 g/h	30	3.5	20% COD	Kalyanaraman et al. (2015)
Acid Black 52 dye (1159 mg/L) - synthetic	-	4 g/h	17.1	4.8	59.8% COD	Vedaraman et al. (2013)
Tannery effluent treatment plant	250.00–700.00	3 g/h	30	3	34.9% COD	Srinivasan et al. (2012)
				12	98% Color	

Figures 5 and 6 shows the results of absolute germination and germination index for all samples.

For the dye samples, according to the toxicity unit classification, samples BA (Beige dye) and PA (Brown dye) fall within Class III (Acute Toxicity). Although both belong to the same class, BA is significantly less toxic than PA. In contrast, sample NO (Walnut dye) falls within Class IV (High acute toxicity). Regarding the germination index, growth was observed at all evaluated concentrations, except for NO, which showed no growth at 100%. Sample BA was classified as category "B" (no significant effect on growth) at 1% and 10%, category "C" (slight growth inhibition) at 3% and 30%, and category "D" (significant growth inhibition) at 100%. Although an unusual fluctuation in toxicity is observed between 3% and 10%, this may be attributed to potential contamination or variability in the 3% sample; however, the change is not abrupt, and BA is consistently the least toxic among the evaluated dyes. Sample PA was classified as category "A" (growth stimulation) at 1%, category "B" at 3% and 10%, and category "D" at 30% and 100%. For sample NO, it was classified as category "C" at 1%, 3%, and 10%, and category "D" at higher concentrations.

The higher toxicity observed for NO, prepared from *Juglans neotropica* leaves, can be explained by the presence of juglone and other phenolic constituents with documented allelopathic activity (Medic et al., 2021). It has also been reported that walnut leaf extracts can affect both germination and early seedling growth, although the magnitude and direction of the effect depend on plant species, concentration, and extract composition. In *L. sativa*, Maksimović and Hasanagić (2020) reported that aqueous leaf extracts inhibited germination and that lettuce was more sensitive than tomato. Furthermore, Medic et al. (2021), showed that *J. regia* leaf extract does not behave identically to pure juglone, suggesting that other extractable compounds contribute to the phytotoxic response; additionally, early growth may be more sensitive than germination.

This interpretation is further supported by biological evidence reported by Babula et al. (2014), which demonstrated that in lettuce roots, juglone inhibits mitosis, reduces meristematic activity, and induces overproduction of reactive oxygen species, lipid peroxidation, alteration of mitochondrial membrane potential, increased intracellular calcium, DNA fragmentation, and programmed cell death. Together, these effects provide a strong biological basis for the marked reduction in germination index observed for sample NO. Moreover, since the leaf extract contains other phenolic compounds in addition to juglone, the higher phytotoxicity of the walnut dye is likely not solely associated with the plant chromophore, but also with the retention of intrinsically bioactive metabolites present in the leaf matrix.

Therefore, one of the most relevant findings of this study is that the natural dye derived from walnut leaves exhibited higher phytotoxicity than the two evaluated synthetic dyes. This result should not be interpreted as contradictory. On one hand, *Juglans regia* leaf extracts have demonstrated direct allelopathic effects on *Lactuca sativa*. On the other hand, the toxicity of synthetic dyes strongly depends on the specific compound, the test organism, and the response evaluated. Rodrigues de Oliveira et al. (2018) studied two synthetic textile dyes, including the azo dye Direct Black 38, and found that although they could produce acute toxicity and/or genotoxicity in aquatic organisms (D. magna) and fish cells (rainbow trout), they did not exhibit phytotoxicity in germination and root elongation assays with *Lactuca sativa*, *Cucumis sativus*, and *Lycopersicon esculentum*. Therefore, the fact that BA and PA were less phytotoxic than NO does not imply that they are environmentally harmless, but rather that under these experimental conditions and for the response of this species, they showed a lower capacity to affect germination and early growth of *Lactuca sativa* seeds.

Table 8. Results of toxicity acute bioassay on *Lactuca sativa* seeds for samples of dyes

Samples	Volume of sample in relation to control hard water volume (%)										EC_{50} (%)	Toxic units
	1		3		10		30		100			
	AG	GI	AG	GI	AG	GI	AG	GI	AG	GI		
BA	0.93	0.87	0.95	0.77	0.97	0.86	0.95	0.61	0.93	0.17	54.89	1.82
PA	0.95	1.26	0.97	0.93	0.98	0.80	0.95	0.24	0.90	0.09	21.02	4.76
NO	0.95	0.57	0.93	0.73	1.00	0.40	1.00	0.34	0.00	0.00	5.21	19.19

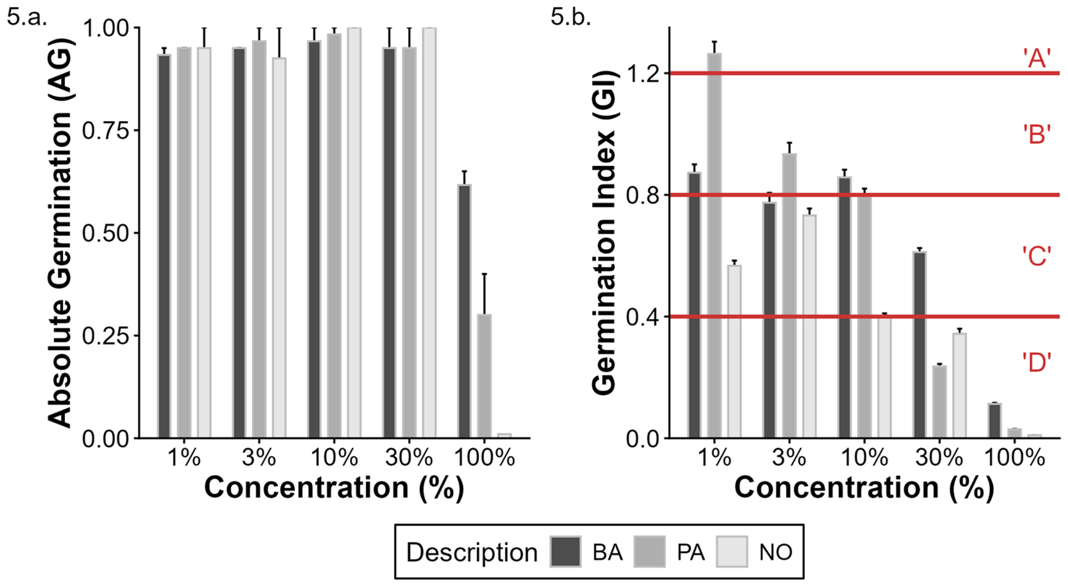

Figure 5. AG and GI for *Lactuca sativa* toxicity test on samples of dyes: (a) absolute germination for each sample at different concentrations – the error bars show the minimum and maximum values observed, (b) germination index for each sample at different concentrations with GI classification limits – the error bars represent the mean ± standard error

For the effluent samples, according to the toxicity unit classification, raw effluent (MI) falls within Class IV, samples EC330 and EC530 fall within Class III, and sample EC730 falls within Class IV. Regarding the germination index analysis, none of the samples showed germination at concentrations above 10%. At the concentrations where growth was observed, sample MI was classified as category "C" at 1% and 3%, and category "D" at 10%. Sample EC330 exhibited lower toxicity at 1%, falling into category "B", and at higher concentrations it was classified as category "C". Samples EC530 and EC730 showed similar behavior and were classified as category "C" across all evaluated concentrations.

The raw effluent exhibited higher phytotoxicity toward *Lactuca sativa*, while electrocoagulation reduced this toxicity in all three tested treatments. However, detoxification was not complete, as all treated samples maintained total inhibition at the highest concentrations (30–100%). The most relevant finding is that the optimal treatment in terms of COD removal (EC730) did not coincide with the treatment showing the lowest toxicity, which was EC330. Therefore, the reduction in organic load did not translate linearly into an equivalent reduction in phytotoxicity. This behavior is consistent with that reported by Palácio et al. (2009), who observed that the lowest toxicity in a textile effluent treated by electrocoagulation was achieved at short electrolysis times (5 min), whereas higher toxicity levels persisted at longer treatment times. Similarly, in tannery effluents treated by electrocoagulation and electrocoagulation–ozonation, it has been observed that, despite reductions in COD and other physicochemical parameters, residual toxicity may persist or even increase after subsequent treatments, a phenomenon attributed to the persistence of toxic compounds and the possible formation of by-products (Aguilar-Ascón et al., 2025).

In this context, a plausible interpretation is that electrocoagulation preferentially removed the coagulable or adsorbable fraction of organic matter, while a dissolved fraction with residual phytotoxic potential remained in solution. This interpretation is consistent with the principles of electrocoagulation, where contaminant removal largely depends on their interaction with electro-generated flocs of metal hydroxides and oxyhydroxides through mechanisms such as adsorption, coprecipitation, charge neutralization, and entrapment. In complex matrices such as tannery effluents, the persistence of dissolved organic matter and compounds less susceptible to coagulation may limit the correspondence between COD removal and the reduction of biological toxicity (Tegladza et al., 2021).

Another possible explanation for toxicity, is that during the electrocoagulation process with aluminum electrodes, aluminum is generated in

Table 9. Results of toxicity acute bioassay on *Lactuca sativa* seeds for samples of effluents

Samples	Volume of sample in relation to control hard water volume (%)											
	1		3		10		30		100		EC_{50} (%)	Toxic units
	AG	GI	AG	GI	AG	GI	AG	GI	AG	GI		
MI	1.00	0.68	0.95	0.62	1.00	0.35	0.00	0.00	0.00	0.00	5.97	16.75
EC330	1.00	0.83	0.93	0.44	1.00	0.69	0.00	0.00	0.00	0.00	22.16	4.51
EC530	1.00	0.71	0.98	0.71	0.98	0.58	0.00	0.00	0.00	0.00	17.03	5.87
EC730	1.00	0.74	0.98	0.65	1.00	0.50	0.00	0.00	0.00	0.00	9.99	10.01

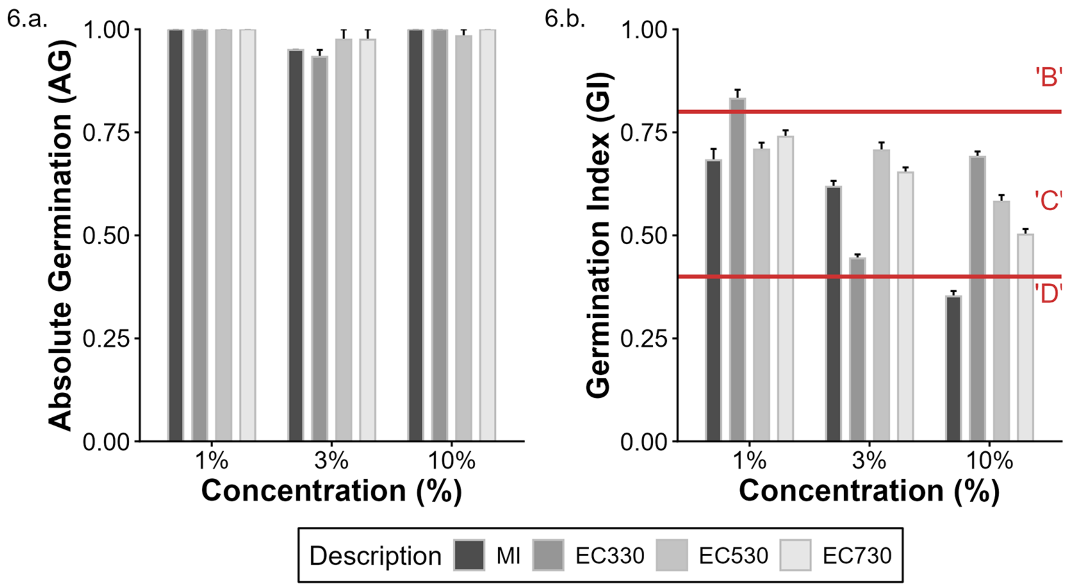

Figure 6. AG and GI for *Lactuca sativa* toxicity test on samples of effluents: (a) absolute germination for each sample at different concentrations – the error bars show the minimum and maximum values observed, (b) germination index for each sample at different concentrations with GI classification limits – the error bars represent the mean ± standard error

its ionic form (Al^{3+}) at the anode. According to Costa da Silva (2014), this form of aluminum inhibits root growth in plants. Consequently, the electrocoagulation-treated effluent would show a higher inhibition coefficient in germination bioassays than the raw effluent due to the presence of bioavailable aluminum or to the formation of reactive chemical species generated during treatment.

According to Charles et al. (2011), treated effluents may still contain contaminant compounds capable of producing toxic effects in aquatic organisms, even if they comply with the limits established by environmental regulations. In the case of this research, dissolved organic matter would remain after electrocoagulation. In line with these results, Sánchez Meza et al. (2007) state that conventional physicochemical parameters are not sufficient to predict the ecological impact of industrial discharges, and that bioassays are therefore required to adequately evaluate the possible adverse effects of treated effluents before final discharge.

It should be noted that Aguilar-Ascón et al. (2024), evaluated a combined electrocoagulation and ozonation system, concluding that the hybrid treatment achieved an effluent with lower toxicity. In that study, a higher germination index of *Lactuca sativa* was also observed in bioassays performed with effluents treated by the hybrid system compared with those treated only by electrocoagulation, indicating a reduction of toxic compounds in the effluent treated by the system that includes ozonation. Nevertheless, the recalcitrant COD associated with walnut dyeing, as shown in this study, may give rise to residual toxicity that should be further investigated.

It is important to note that the obtained results suggest the need to move toward more comprehensive ecotoxicological assessments of treated effluents. In particular, it would be appropriate to incorporate chronic toxicity assays, since acute effects do not necessarily reflect sublethal effects resulting from prolonged exposure. Likewise, the comparison between wastewater treatments from dyeing processes using natural and synthetic dyes should be expanded through batteries of bioassays at different trophic levels, as the origin of the dye alone does not predict lower environmental hazard, and the reduction of physicochemical parameters does not always imply a homogeneous reduction in toxicity. This approach would allow the generation of more robust criteria for both input substitution and treatment selection.

CONCLUSIONS

The development of sustainable alternatives, such as replacing synthetic dyes with natural dyes, involves technical challenges related not only to the functional performance of the final leather products but also to the environmental impacts generated throughout processing. In this context, leather processing technologies for Amazonian species such as paiche should be approached from a holistic perspective, in which product performance and effluent management are considered simultaneously.

In the present study, electrocoagulation of effluents generated during the dyeing of paiche skins with a walnut-based dye showed the best performance at 7 A and 30 min, achieving a COD removal of 51.67%. A subsequent ozonation step, operated at 1 g/h, pH of 5, and 120 min of contact time, provided an additional COD removal of 12.51%. Toxicity assays using Lactuca sativa revealed a toxic effect associated with the walnut-based dye, attributed to its active compound, juglone. For the effluents, the treatment reduced toxicity compared to the raw effluent; however, an increase in toxicity was observed with increasing current intensity.

These findings indicate that the combined use of electrocoagulation and ozonation is a promising strategy for reducing the organic pollution load of this type of effluent, although important limitations remain. In particular, further research is needed to develop hybrid treatment systems capable of improving overall treatment efficiency and enabling water reuse, while also controlling treatment-associated toxicity that is not captured by the physicochemical parameters currently considered in environmental regulations.

In addition, scale-up will depend on the availability of technical and economic resources. Although paiche aquaculture in Peru is still developing, the high added value of paiche leather supports the existence of a niche market that justifies continued research into cleaner processing technologies, including the use of agricultural and agro-industrial by-products as alternatives to synthetic chemical inputs.

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REFERENCES

1. Aguilar-Ascón, E., Marrufo-Saldaña, L., Barra-Hinojosa, J. A. (2024). Combining effluent treatment methods to remove ammonia nitrogen from tannery wastewater. *Ecological Engineering & Environmental Technology*, *25*(12), 371–386. https://doi.org/10.12912/27197050/194949
2. Aguilar-Ascón, E., Marrufo-Saldaña, L., Barra-Hinojosa, J., Buleje-del-Carpio, R. (2025). Assessment of tannery effluents quality treated by electrocoagulation and ozonation: Physicochemical and ecotoxicological characterization. *PLOS One*, *20*(7), e0328654. https://doi.org/10.1371/journal.pone.0328654
3. Alegbe, E. O., Uthman, T. O. (2024). A review of history, properties, classification, applications and challenges of natural and synthetic dyes. *Heliyon*, *10*(13), e33646. https://doi.org/10.1016/j.heliyon.2024.e33646
4. Ardhan, N., Tongpadungrod, P., Phalakornkule, C. (2022). Effects of auxiliary chemicals and dye solubility on chemical oxygen demand reduction of dyes by electrocoagulation with Fe electrode. *Materials Today: Proceedings*, *52*, 2529–2533. https://doi.org/10.1016/j.matpr.2021.10.446

5. Ardila-Leal, L. D., Poutou-Piñales, R. A., Pedroza-Rodríguez, A. M., Quevedo-Hidalgo, B. E. (2021). A brief history of colour, the environmental impact of synthetic dyes and removal by using laccases. *Molecules*, *26*(13), 3813. https://doi.org/10.3390/molecules26133813
6. Aryanti, P. T. P., Nugroho, F. A., Phalakornkule, C., Kadier, A. (2024). Energy efficiency in electrocoagulation processes for sustainable water and wastewater treatment. *Journal of Environmental Chemical Engineering*, *12*(6), 114124. https://doi.org/10.1016/j.jece.2024.114124
7. Aygun, A., Nas, B., Sevimli, M. F. (2021). Electrocoagulation of disperse dyebath wastewater: optimization of process variables and sludge production. *Journal of Electrochemical Science and Technology*, *12*(1), 82–91. https://doi.org/10.33961/jecst.2020.00787
8. Babula, P., Vaverkova, V., Poborilova, Z., Ballova, L., Masarik, M., Provaznik, I. (2014). Phytotoxic action of naphthoquinone juglone demonstrated on lettuce seedling roots. *Plant Physiology and Biochemistry*, *84*, 78–86. https://doi.org/https://doi.org/10.1016/j.plaphy.2014.08.027
9. Balakrishnan, A., Kanchinadham, S. B. K., Kalyanaraman, C. (2020). Studies on the effect of pretreatment of vegetable tanning process wastewater prior to biological treatment. *Journal of Environmental Chemical Engineering*, *8*(4), 104020. https://doi.org/10.1016/j.jece.2020.104020
10. Barra-Hinojosa, J., Marrufo-Saldaña, L. (2020). Optimization of alkaline hydrolysis of chrome shavings to recover collagen hydrolysate and chromium hydroxide. *Leather and Footwear Journal*, *20*(1), 15–28. https://doi.org/10.24264/lfj.20.1.2
11. Başaran Dindaş, G., Çalışkan, Y., Çelebi, E. E., Tekbaş, M., Bektaş, N., Yatmaz, H. C. (2020). Treatment of pharmaceutical wastewater by combination of electrocoagulation, electro-fenton and photocatalytic oxidation processes. *Journal of Environmental Chemical Engineering*, *8*(3), 103777. https://doi.org/10.1016/j.jece.2020.103777
12. Beltran, F. J. (2003). *Ozone Reaction Kinetics for Water and Wastewater Systems*. CRC Press. https://doi.org/10.1201/9780203509173
13. Carrasquero-Ferrer, S. J., Guerra-Núñez, D. G., León-Piñango, L. J., Hernández-González, G. A. (2024). Eficiencia de un tratamiento aeróbico para la remoción de materia orgánica en efluentes de origen textil usando reactores biológicos. *Revista de Gestão Social e Ambiental*, *18*(6), e05506. https://doi.org/10.24857/rgsa.v18n6-091
14. Charles, J., Sancey, B., Morin-Crini, N., Badot, P.-M., Degiorgi, F., Trunfio, G., Crini, G. (2011). Evaluation of the phytotoxicity of polycontaminated industrial effluents using the lettuce plant (*Lactuca sativa*) as a bioindicator. *Ecotoxicology and Environmental Safety*, *74*(7), 2057–2064. https://doi.org/10.1016/j.ecoenv.2011.07.025
15. Costa da Silva, A. (2014). Maize growth in Aluminum-rich Vertisoils from the lowland pre-Amazonia, Baixada Maranhense, Brazil. *Agronomía Mesoamericana*, *25*(2), 367–370. https://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S1659-13212014000200014
16. Das, P. P., Dhara, S., Samanta, N. S., Purkait, M. K. (2024). Advancements on ozonation process for wastewater treatment: A comprehensive review. *Chemical Engineering and Processing - Process Intensification*, *202*, 109852. https://doi.org/10.1016/j.cep.2024.109852
17. Erkan, G., Şengül, K., Kaya, S. (2014). Dyeing of white and indigo dyed cotton fabrics with Mimosa tenuiflora extract. *Journal of Saudi Chemical Society*, *18*(2), 139–148. https://doi.org/10.1016/j.jscs.2011.06.001
18. Farhan Abbass, R., Amooey, A. A., Yasir AlJaberi, F. (2024). Electrocoagulation removal of COD and TDS from real municipal wastewater sourced from the Euphrates River using multipole arrangement. *Results in Chemistry*, *9*, 101613. https://doi.org/10.1016/j.rechem.2024.101613
19. Garcia-Segura, S., Eiband, M. M. S. G., de Melo, J. V., Martínez-Huitle, C. A. (2017). Electrocoagulation and advanced electrocoagulation processes: A general review about the fundamentals, emerging applications and its association with other technologies. *Journal of Electroanalytical Chemistry*, *801*, 267–299. https://doi.org/10.1016/j.jelechem.2017.07.047
20. Gasmi, A., Ibrahimi, S., Elboughdiri, N., Tekaya, M. A., Ghernaout, D., Hannachi, A., Mesloub, A., Ayadi, B., Kolsi, L. (2022). Comparative study of chemical coagulation and electrocoagulation for the treatment of real textile wastewater: Optimization and operating cost estimation. *ACS Omega*, *7*(26), 22456–22476. https://doi.org/10.1021/acsomega.2c01652
21. Ghernaout, D., Alghamdi, A., Ghernaout, B. (2019). Electrocoagulation process: A mechanistic review at the dawn of its modeling. *Journal of Environmental Science and Allied Research*, *2*(1), 22–38. https://doi.org/10.29199/2637-7063/ESAR-201019
22. Gómez, C. A., Gómez-García, M.-Á., Dobrosz-Gómez, I. (2023). Analysis of the capacity of the fenton process for the treatment of polluted wastewater from the leather dyeing industry. *The Scientific World Journal*, *2023*, 1–21. https://doi.org/10.1155/2023/4724606
23. Hansen, É., Monteiro de Aquim, P., Gutterres, M. (2021). Current technologies for post-tanning wastewater treatment: A review. *Journal of Environmental Management*, *294*, 113003. https://doi.org/10.1016/j.jenvman.2021.113003

24. Jiménez-Pacheco, H. G., Meza-Elguera, N. Y. M., Quintanilla-Sarka, H. D., Ancco, M., Barrios-Eguiluz, K. I., Salazar-Banda, G. R. (2022). Box-Behnken response surface design for modeling and optimization of electrocoagulation for treating real textile wastewater. *International Journal of Environmental Research*, *16*(4), 43. https://doi.org/10.1007/s41742-022-00419-4
25. Jing, G., Ren, S., Gao, Y., Sun, W., Gao, Z. (2020). Electrocoagulation: A promising method to treat and reuse mineral processing wastewater with high COD. *Water*, *12*(2), 595. https://doi.org/https://doi.org/10.3390/w12020595
26. Kabdaşlı, I., Arslan-Alaton, I., Ölmez-Hancı, T., Tünay, O. (2012). Electrocoagulation applications for industrial wastewaters: a critical review. *Environmental Technology Reviews*, *1*(1), 2–45. https://doi.org/10.1080/21622515.2012.715390
27. Kalyanaraman, C., Kameswari, K. S. B., Rao, J. R. (2015). Studies on enhancing the biodegradation of tannins by ozonation and Fenton's oxidation process. *Journal of Industrial and Engineering Chemistry*, *25*, 329–337. https://doi.org/10.1016/j.jiec.2014.11.012
28. Kasiri, M. B., Modirshahla, N., Mansouri, H. (2013). Decolorization of organic dye solution by ozonation; Optimization with response surface methodology. *International Journal of Industrial Chemistry*, *4*(1), 3. https://doi.org/10.1186/2228-5547-4-3
29. Kermet-Said, H., Moulai-Mostefa, N. (2015). Optimization of turbidity and COD removal from pharmaceutical wastewater by electrocoagulation. Isotherm modeling and cost analysis. *Polish Journal of Environmental Studies*, *24*, 1049–1061. https://doi.org/10.15244/pjoes/32334
30. Khelali, A., Benmahdi, F., Sahnoune, D., Sami, K., Hacıosmanoğlu, G. G., Akram, S., Isinkaralar, K., Siswanta, D., Hosseini-Bandegharaei, A. (2025). Removal of AG 25 dye from aqueous solutions and treatment of real tannery wastewater by reusable magnetic iron oxide nanoparticles loaded with pomegranate pomace extract. *Environmental Research*, *279*, 121744. https://doi.org/10.1016/j.envres.2025.121744
31. Khosravi, R., Hazrati, S., Fazlzadeh, M. (2016). Decolorization of AR18 dye solution by electrocoagulation: sludge production and electrode loss in different current densities. *Desalination and Water Treatment*, *57*(31), 14656–14664. https://doi.org/10.1080/19443994.2015.1063092
32. Kraljić Roković, M., Čubrić, M., Wittine, O. (2014). Phenolic compounds removal from mimosa tannin model water and olive mill wastewater by energy-efficient electrocoagulation process. *Journal of Electrochemical Science and Engineering*, *4*(4). https://doi.org/10.5599/jese.2014.0066
33. Lama-Muñoz, A., Contreras, M. del M. (2022). Extraction systems and analytical techniques for food phenolic compounds: A review. *Foods*, *11*(22), 3671. https://doi.org/10.3390/foods11223671
34. Lanzetta, A., Papirio, S., Oliva, A., Cesaro, A., Pucci, L., Capasso, E. M., Esposito, G., Pirozzi, F. (2023). Ozonation processes for color removal from urban and leather tanning wastewater. *Water*, *15*(13), 2362. https://doi.org/10.3390/w15132362
35. Lim, S., Shi, J. L., von Gunten, U., McCurry, D. L. (2022). Ozonation of organic compounds in water and wastewater: A critical review. *Water Research*, *213*, 118053. https://doi.org/10.1016/j.watres.2022.118053
36. Maksimović, T., Hasanagić, D. (2020). Allelopathic influence of *Juglans regia* L. aqueous extract germination and growth of lettuce and tomato. *Journal of Agricultural, Food and Environmental Sciences*, *74*(2). https://doi.org/https://doi.org/10.55302/JAFES20742005m
37. Malik, S. N., Ghosh, P. C., Vaidya, A. N., Mudliar, S. N. (2020). Hybrid ozonation process for industrial wastewater treatment: Principles and applications: A review. *Journal of Water Process Engineering*, *35*, 101193. https://doi.org/10.1016/j.jwpe.2020.101193
38. Márquez, A. A., Coreño, O., Nava, J. L. (2022). Removal of brilliant green tannery dye by electrocoagulation. *Journal of Electroanalytical Chemistry*, *911*, 116223. https://doi.org/10.1016/j.jelechem.2022.116223
39. Marrufo-Saldaña, L., Lopez-Alvarez, A., Sueldo-Rojas, H., Laura-Tito, M., Barra-Hinojosa, J., Uribe-Valenzuela, C. (2024). Evaluation of the dyeing process for paiche (*Arapaima Gigas*) leather using colorant extracted from walnut leaves (*Juglans Neotropica*) for scaling-up. *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, *43*(9), 3306–3319. https://doi.org/10.30492/ijcce.2024.2020264.6410
40. Masias-Bröcker, K. (2007). *Caracterización de las propiedades tintóreas del extracto de nogal (Juglans neotropica Diles) proveniente de la cuenca alta del río Zaña*. Universidad Nacional Agraria La Molina, Peru.
41. Medic, A., Zamljen, T., Slatnar, A., Hudina, M., Veberic, R. (2021). Is juglone the only naphthoquinone in *Juglans regia* L. with allelopathic effects? *Agriculture*, *11*(8), 784. https://doi.org/https://doi.org/10.3390/agriculture11080784
42. Moussa, D. T., El-Naas, M. H., Nasser, M., Al-Marri, M. J. (2017). A comprehensive review of electrocoagulation for water treatment: Potentials and challenges. *Journal of Environmental Management*, *186*, 24–41. https://doi.org/10.1016/j.jenvman.2016.10.032

43. Nandi, B. K., Patel, S. (2013). Effects of operational parameters on the removal of brilliant green dye from aqueous solutions by electrocoagulation. *Arabian Journal of Chemistry*, *249*(S2). https://doi.org/10.1016/j.arabjc.2013.11.032
44. New York Post. (2022). Giant sustainable rainforest fish now fashion in America. *New York Post*. https://nypost.com/2022/11/04/giant-sustainable-rainforest-fish-now-fashion-in-america/
45. Nguyen, D. H., Nguyen, V. B., Nguyen, D. B., Nguyen, C. T. (2012). *Tree allometric equation development for estimation of forest above-ground biomass in Viet Nam -- Evergreen broadleaf, Deciduous, and Bamboo forests in the South East region*. https://www.un-redd.org/document-library/part-b-5-tree-allometric-equations-evergreen-broadleaf-deciduous-and-bamboo
46. Ojstršek, A., Fakin, D. (2019). Natural dyeing of wool using *Junglans regia* (Common Walnut) leaf extract. *TEKSTILEC*, *62*(4), 288–298. https://doi.org/10.14502/Tekstilec2019.62.288-298
47. Ojstršek, A., Vouk, P., Fakin, D. (2022). Adsorption of pollutants from colored wastewaters after natural wool dyeing. *Materials*, *15*(4), 1488. https://doi.org/10.3390/ma15041488
48. Ortiz-Monsalve, S. (2017). *Treatment of tannery dye-containing effluents using a native fungal strain*.
49. Palácio, S. M., Espinoza-Quiñones, F. R., Módenes, A. N., Oliveira, C. C., Borba, F. H., Silva, F. G. (2009). Toxicity assessment from electro-coagulation treated-textile dye wastewaters by bioassays. *Journal of Hazardous Materials*, *172*(1), 330–337. https://doi.org/10.1016/j.jhazmat.2009.07.015
50. Postigo-Sobrevilla, Pacheco-Roldán, D., Vásquez-Vizarreta, C., Castro-Daza, F., Zagarra-Adrianzén, R., Paima Alayo, G. C. (2023). *Anuario Forestal y de Fauna Silvestre 2023*. https://repositorio.serfor.gob.pe/handle/SERFOR/969
51. Prayochmee, S., Weerayutsil, P., Khuanmar, K. (2021). Response surface optimization of electrocoagulation to treat real indigo dye wastewater. *Polish Journal of Environmental Studies*, *30*(3), 2265–2271. https://doi.org/10.15244/pjoes/127388
52. Preethi, V., Parama Kalyani, K. S., Iyappan, K., Srinivasakannan, C., Balasubramaniam, N., Vedaraman, N. (2009). Ozonation of tannery effluent for removal of cod and color. *Journal of Hazardous Materials*, *166*(1), 150–154. https://doi.org/10.1016/j.jhazmat.2008.11.035
53. R Core Team. (2025). *R: A Language and Environment for Statistical Computing* (R 4.5.2). https://www.r-project.org/
54. Rendón-Castrillón, L., Ramírez-Carmona, M., Ocampo-López, C., González-López, F., Cuartas-Uribe, B., Mendoza-Roca, J. A. (2023). Treatment of water from the textile industry contaminated with indigo dye: A hybrid approach combining bioremediation and nanofiltration for sustainable reuse. *Case Studies in Chemical and Environmental Engineering*, *8*, 100498. https://doi.org/10.1016/j.cscee.2023.100498
55. Rodrigues de Oliveira, G. A. R. de, Leme, D. M., de Lapuente, J., Brito, L. B., Porredón, C., Rodrigues, L. de B., Brull, N., Serret, J. T., Borràs, M., Disner, G. R., Cestari, M. M., Oliveira, D. P. de. (2018). A test battery for assessing the ecotoxic effects of textile dyes. *Chemico-Biological Interactions*, *291*, 171–179. https://doi.org/10.1016/j.cbi.2018.06.026
56. Saha, R. K., Paula, H., Hashem, M. A., Hira, J. (2025). Investigation of dye adsorption on thermally activated adsorbent derived from Tamarindus indica leaves. *Journal of Engineering Science*, *15*(2), 105–112. https://doi.org/10.3329/jes.v15i2.82166
57. Saira, G. C., Shanthakumar, S. (2023). Zero waste discharge in tannery industries – An achievable reality? A recent review. *Journal of Environmental Management*, *335*, 117508. https://doi.org/10.1016/j.jenvman.2023.117508
58. Sánchez-Meza, J. C., Pacheco-Salazar, V. F., Pavón-Silva, T. B., Guiérrez-García, V. G., De Jesús Avila-González, C., Guerrero-García, P. (2007). Toxicity assessment of a complex industrial wastewater using aquatic and terrestrial bioassays *Daphnia pulex* and *Lactuca sativa*. *Journal of Environmental Science and Health, Part A*, *42*(10), 1425–1431. https://doi.org/10.1080/10934520701480730
59. Sankar, M. S. R., Sivasubramanian, V., Vijay, E. V. V., Jerold, M., Kanimozhi, J., Sinu, P., Shankar, N. (2018). Kinetic, isothermal and thermodynamic investigation on electrocoagulation of Congo red dye removal from synthetic wastewater using aluminium electrodes. *Desalination and Water Treatment*, *122*, 339–350. https://doi.org/10.5004/dwt.2018.23082
60. Santos, A., Barros, L., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., Ferreira, I. C. F. R. (2013). Leaves and decoction of *Juglans regia* L.: Different performances regarding bioactive compounds and in vitro antioxidant and antitumor effects. *Industrial Crops and Products*, *51*, 430–436. https://doi.org/10.1016/j.indcrop.2013.10.003
61. Segundo Espada, A., Marrufo Saldaña, L., Barra Hinojosa, J., Contreras Panizo, R. (2020). Development of a degreasing process for paiche skins *(Arapaima gigas*) for tanning preserving the natural pattern and color. *Leather and Footwear Journal*, *20*(2), 119–132. https://doi.org/10.24264/lfj.20.2.3
62. Shaker, A. M., Moneer, A. A., El-Sadaawy, M. M., El-Mallah, N. M., Ramadan, M. S. H. (2020). Comparative study for removal of acid green 20 dye by electrocoagulation technique using aluminum and iron electrodes. *Desalination and Water Treatment*, *198*, 345–363. https://doi.org/10.5004/dwt.2020.26007

63. Slama, H. Ben, Chenari Bouket, A., Pourhassan, Z., Alenezi, F. N., Silini, A., Cherif-Silini, H., Oszako, T., Luptakova, L., Golińska, P., Belbahri, L. (2021). Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. *Applied Sciences*, *11*(14), 6255. https://doi.org/10.3390/app11146255
64. Sójka, M., Janowski, M., Grzelak-Błaszczyk, K. (2019). Stability and transformations of raspberry (Rubus idaeus L.) ellagitannins in aqueous solutions. *European Food Research and Technology*, *245*(5), 1113–1122. https://doi.org/10.1007/s00217-018-3212-3
65. Solayman, H. M., Hossen, M. A., Abd Aziz, A., Yahya, N. Y., Leong, K. H., Sim, L. C., Monir, M. U., Zoh, K.-D. (2023). Performance evaluation of dye wastewater treatment technologies: A review. *Journal of Environmental Chemical Engineering*, *11*(3), 109610. https://doi.org/10.1016/j.jece.2023.109610
66. Srinivasan, S. V., Mary, G. P. S., Kalyanaraman, C., Sureshkumar, P. S., Sri Balakameswari, K., Suthanthararajan, R., Ravindranath, E. (2012). Combined advanced oxidation and biological treatment of tannery effluent. *Clean Technologies and Environmental Policy*, *14*(2), 251–256. https://doi.org/10.1007/s10098-011-0393-x
67. Strugstad, M., Despotovski, S. (2013). A Summary of extraction, synthesis, properties, and potential uses of juglone: A literature review. *Journal of Ecosystems and Management*, *13*(3). https://doi.org/10.22230/jem.2012v13n3a119
68. Sugha, A., Bhatti, M. S. (2025). Optimization of electrocoagulation removal of a mixture of three azo dyes: spectrophotometric colour characteristics for best operating conditions. *RSC Advances*, *15*(9), 6492–6505. https://doi.org/10.1039/D4RA08485C
69. Suman, H., Sangal, V. K., Vashishtha, M. (2021). Treatment of tannery industry effluent by electrochemical methods: A review. *Materials Today: Proceedings*, *47*, 1438–1444. https://doi.org/10.1016/j.matpr.2021.03.300
70. Tegladza, I. D., Xu, Q., Xu, K., Lv, G., Lu, J. (2021). Electrocoagulation processes: A general review about role of electro-generated flocs in pollutant removal. *Process Safety and Environmental Protection*, *146*, 169–189. https://doi.org/10.1016/j.psep.2020.08.048
71. Toro Vanegas, E., Roldan Rojas, I. C. (2018). Estado del arte, propagación y conservación de Juglans neotropica Diels., en zonas andinas. *Madera y Bosques*, *24*(1). https://doi.org/10.21829/myb.2018.2411560
72. Toscanesi, M., Russo, V., Medici, A., Giarra, A., Hmoudah, M., Di Serio, M., Trifuoggi, M. (2022). Heterogeneous photodegradation for the abatement of recalcitrant COD in synthetic tanning wastewater. *ChemEngineering*, *6*(2), 25. https://doi.org/10.3390/chemengineering6020025
73. Vedaraman, N., Shamshath Begum, S., Srinivasan, S. V. (2013). Response surface methodology for decolourisation of leather dye using ozonation in a packed bed reactor. *Clean Technologies and Environmental Policy*, *15*(4), 607–616. https://doi.org/10.1007/s10098-012-0544-8
74. Venkataraman, A., Babu, L., Aravamudan, K. (2022). Unified, simple and decentralized treatment process for synthetic and real-time dye contaminated wastewaters. *Journal of Hazardous Materials*, *423*, 127059. https://doi.org/10.1016/j.jhazmat.2021.127059
75. Villaseñor-Basulto, D. L., Kadier, A., Singh, R., Navarro-Mendoza, R., Bandala, E., Peralta-Hernández, J. M. (2022). Post-tanning wastewater treatment using electrocoagulation: Optimization, kinetics, and settlement analysis. *Process Safety and Environmental Protection*, *165*, 872–886. https://doi.org/10.1016/j.psep.2022.08.008
76. von Gunten, U. (2003). Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. *Water Research*, *37*(7), 1469–1487. https://doi.org/10.1016/S0043-1354(02)00458-X
77. Yadav, S., Tiwari, K. S., Gupta, C., Tiwari, M. K., Khan, A., Sonkar, S. P. (2023). A brief review on natural dyes, pigments: Recent advances and future perspectives. *Results in Chemistry*, *5*, 100733. https://doi.org/10.1016/j.rechem.2022.100733
78. Zafar, A. M., Naeem, A., Minhas, M. A., Hasan, M. J., Rafique, S., Ikhlaq, A. (2024). Removal of reactive dyes from textile industrial effluent using electrocoagulation in different parametric conditions of aluminum electrodes. *Total Environment Advances*, *9*, 200087. https://doi.org/10.1016/j.teadva.2023.200087