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Assessment of the Hazard and Ecotoxicity of the Residues of Paiche Leather Dyed with Natural and Synthetic Colourants: A Focus on Environmental Sustainability

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Article

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ABSTRACT

The aim of this research was to assess the hazardousness and ecotoxicity of paiche leather (Arapaima gigas) dyed with natural colourants (turmeric and purple corn) versus synthetic colourants (anilines). To determine hazardousness, the methodology included the assessment of flammability, corrosivity, reactivity to H2S and HCN, toxicity from metals, and organic compounds of dyed leather. Additionally, its ecotoxicity was determined using the California red worm (Eisenia fetida) as a bioindicator through a bioassay, with treatments at 0.02, 0.04, 0.08, 0.16, and 0.20 g of leather/g of substrate, including undyed leather and a negative control in the investigation. The hazardousness analysis results indicated that leather samples dyed with both natural and synthetic colourants are not hazardous. However, the concentrations of chromium, nickel, cadmium, and lead exceed the permitted values for their use in footwear according to the European Union. Regarding ecotoxicity, the results demonstrate that acute toxicity to E. fetida is lower for natural colourants compared to synthetic colourants, where 100% mortality occurs at concentrations between 0.04 and 0.08 g of leather/g of the substrate. Concerning chronic toxicity, after 12 weeks of exposure, the tolerance concentration for undyed paiche leather is 0.035 g of leather/g of the substrate, and for leather dyed with purple corn and turmeric, it is 0.2 g of leather/g of the substrate. It is shown that the active compounds of natural colourants have a positive effect on worm survival, and it is concluded that paiche leather dyed with these colourants presents environmental advantages over leather dyed with synthetic colourants.

KEYWORDS

paiche, leather, residues, toxicity, Eisenia, natural dyes, synthetic dyes

INTRODUCTION

The tanning industry, which traditionally focused on tanning hides and skins of cattle, sheep, goats, and pigs, has now expanded its horizons under conditions of permissible breeding and/or hunting, gaining access to exotic hides and skins of high commercial value [1,2]. Among these raw materials for leather production, are skins from a wide variety of fish such as paiche, whose productive activity is mainly promoted in Brazil, Peru, and Colombia [3].

In the leather industry, colour plays a crucial role in a consumer's purchasing decision. Over time, with the technological advancement of synthetic colourants and chromium tanning, dyeing technology has seen significant development [4]. Among the attributes that have led to a preference for synthetic colourants, their greater resistance to rubbing and light, as well as their vibrant and deep tones, can be cited [4].

According to application, synthetic colourants used in the leather industry can be classified as acid, basic, direct, metallised, reactive, and sulphur dyes [5]. The main functional groups present in these dyes are azo, anthraquinone, aromatic methane, reducing polynuclear ketone, phthalocyanine, sulfur, thiazole, and acridine, among others. Acid dyes are the most commonly used in the leather industry; many of them are monoazo and diazo, water-soluble, light-stable, highly colourful, have high penetrability, and are resistant to bacterial degradation [6,7].

Azoic colourants are compounds formed by the functional group azo chromophore (-N=N-), joining two symmetrical and/or asymmetrical identical or non-azo alkyl or aryl radicals. The toxicity of some azoic colourants has been demonstrated either by themselves or by the aromatic amines generated as a product of their decomposition [6,8]. Reported toxic effects of azoic colourants include lethality, genotoxicity, mutagenicity, carcinogenicity, and teratogenicity in humans as well as animals [6,9]. Consequently, legislation in various countries has established the prohibition of some of these colourants, as well as traces of associated aromatic amines [8]. REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), the regulation of the European Union (EU) on chemicals, has identified 22 aromatic amines that are prohibited in textiles and leather at a concentration higher than 30 ppm. Additionally, if the articles are made of recycled fibres, they cannot contain more than 70 ppm [10].

Exposure routes to azoic colourants for humans can include ingestion, inhalation, and dermal contact. In the case of dermal exposure, this can occur through the use of textile articles and leather that come into contact with the skin. It has been demonstrated that certain skin microorganisms may play a role in the degradation of colourants into highly toxic aromatic amines [6].

Furthermore, liquid and solid waste generated in the production of textiles and leather contains concentrations of these compounds that, upon contact with the abiotic and biotic components of ecosystems, compromise their normal functioning [11]. Several toxicity studies have been conducted with bioindicator organisms exposed to azo dyes. Some of these studies have demonstrated that their degradation by microbial consortia can generate aromatic amines that are more toxic than the precursor dye [11]. In plants, the environmental presence of azo compounds has been reported to result in reduced photosynthetic activity, inhibited plant growth, and decreased soil quality [12].

In the tanning industry, there are efforts to achieve technological development in their tanning processes and thus utilize the waste skin sustainably [13]. In this regard, the substitution of synthetic chemicals in the production process with less toxic and more biodegradable alternatives, such as organic tanning agents and colourants, is a current concern [13]. However, despite the perception of lower hazardousness in these substitutes, it is necessary to demonstrate this attribute to enhance the value of the resulting products.

Currently, research on the application of natural colourants in the food, textile, cosmetic, and leather industries has gained significant importance [14]. This responds to the need to develop biodegradable compounds that do not harm ecosystems and do not pose harm to human health [15]. Natural colourants can be extracted from edible plants and their residues [16]. Unlike synthetic colourants, natural colourants are not harmful to humans and do not pose risks to the environment [17]. Additionally, they are renewable, non-toxic, and sustainable, while possessing functional properties such as antibacterial, antifungal, antimicrobial, antioxidant, and repellent qualities. They are successfully utilized in dyeing textiles and leather [18–22]. However, concerns also exist regarding their lightfastness, reproducibility of dyeing processes, and scalability due to raw material availability in terms of volume and seasonality, among other factors [16]. Natural colourants can be classified according to their origin into animal, vegetable, or mineral [22]. In the specific case of leather, various studies indicate that the application of natural colourants in the dyeing process is feasible, maintaining adequate colour fastness. Several studies have reported the extraction and application of natural colourants from plant species in the dyeing of cow, sheep, and goat hides. For example, *Coreopsis tinctoria* flower petals, sweet potato, or black chokeberry have been used [23], as well as *Garcinia mangostana Linn* (*G. mangostana Linn*) skin wastes [24], stem bark of *Trema orientalis (L.)* [25], onion peel (*Allium cepa*) [26], beetroot peels [27], *Cassia Singueana* plant, among others [2].

Agricultural resources generate products and residues containing naturally occurring organic compounds of high value and significant potential for use in the industry. Among these compounds, natural colourants stand out, whose use dates back several centuries. However, they were replaced by synthetic colourants when the latter were synthesized, providing the industry with various advantages, such as stability, a wide range of shades, and increased production volumes [23].

Among plant species with dyeing properties, turmeric and purple corn stand out and due to their widespread applications, these plants have high production levels and have been the focus of numerous initiatives aimed at harnessing and diversifying the benefits of their active compounds [28,29].

Turmeric, scientifically known as *Curcuma longa*, is a herbaceous plant [30]. Its medicinal use dates back over 4.000 years [31]. Currently, its use as both a spice and a natural colourant is prominent [31]. However, its applications are not limited to the food industry but have also extended to the pharmaceutical and cosmetic industries [28].

Among the leading turmeric-producing countries are India, Burma, and the Netherlands [32]. Peru exports turmeric, primarily to countries such as the Netherlands, Spain, and Canada. Exports increased by 5% as of July 2023, reaching a value of 2, 831, 946 FOB dollars [33].

Turmeric contains a variety of components, including carbohydrates, proteins, fats, minerals, and bioactive compounds [34]. Its roots are particularly rich in curcuminoids, which are responsible for the characteristic yellow-orange colour. Several studies have addressed the toxicity of turmeric and curcumin extracts, demonstrating that their use would be safe [35]. Curcuminoids have been approved by the US Food and Drug Administration (FDA) as "generally recognized as safe" [36]. Positive effects have been reported in various aspects of health, including anticancer, anti-inflammatory, and antioxidant properties, as well as benefits in cardiovascular diseases, diabetes, obesity, neurodegenerative diseases, and allergies, among others [30].

Curcuminoids consist of three phenols: curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin [30]. It is important to note that the stability of curcumin depends on the pH. In solutions with a pH lower than 1, curcumin appears red due to the presence of the protonated form. In a pH range of 1 to 7, curcumin solutions are yellow, with the majority of molecules in the neutral form. When the pH exceeds 7.5, curcumin solutions undergo a colour change to reddish-orange [37].

Purple corn (*Zea mays L.*) is a crop native to Peru [38]. Peru has six main varieties of purple corn (Cuzco, Canteño, Caraz, Arequipeño, Negro de Junín, Huancavelicano) and five improved varieties [39]. In August 2023, purple corn exports experienced a 27% increase, generating revenue of 1, 175, 386 million dollars FOB [40]. This cereal contains compounds such as fats, potassium salts, resins, salicylic acid, phosphorus, saponins, sodium, phenols, and sulfur [41]. Among the present phenols, flavonoids are found, with the most important being anthocyanins, which are glycosides of anthocyanidins [42].

Anthocyanins are found in higher concentrations in the cob, which constitutes 15% of the cereal, while in the grains, representing 85% of the composition, small concentrations are present in the husk [39,43]. These substances are responsible for giving colour to corn. Their basic structure contains a flavone core composed of two aromatic rings: a benzopyrillium and a phenolic group, both connected by a three-carbon unit [39].

The stability of anthocyanins depends on the type of anthocyanin pigment, as well as other factors such as pH, light, temperature, enzymes, and other molecules, such as oxygen and ascorbic acid [39]. In aqueous solutions with a pH lower than 3, anthocyanin exhibits a red colour, and the flavan nucleus primarily exists as a very stable flavylium cation. As the pH increases, deprotonation occurs to form the purple quinonoidal base as a kinetic product, leading to the formation of the anionic quinonoidal base at higher pH, presenting a blue colour [44]. Anthocyanins possess pharmacological and therapeutic properties. Scientific evidence supports their anticancer, anti-inflammatory, antitumor, and antidiabetic properties. Additionally, they have been observed to contribute to the reduction of coronary diseases [45–47].

It's worth noting that in the tanning industry, primary environmental concerns revolve around water consumption, the quality and destination of effluents, as well as solid waste management [48–50]. During the tanning process, 80% of the raw material is discarded as solid and liquid waste [51]. In the case of the residues generated in the tanning process, their characteristics are diverse due to the nature of the production process [51]. Therefore, assessing the risks associated with these aspects becomes a key element for the technical-economic evaluation in the implementation of new technologies.

According to the Environmental Protection Agency (EPA), "a waste is hazardous waste if it is specifically listed as a known hazardous waste or meets the characteristics of a hazardous waste". To be classified as hazardous waste, a residue must possess the following characteristics: flammability, corrosivity, reactivity, or toxicity. Solid waste is deemed inflammable when it is "liable to cause fires through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or when ignited burns so vigorously and persistently as to create a hazard during its management". The corrosiveness of solid waste is manifested if a representative sample of the waste is aqueous and has a pH less than or equal to 2 or greater than or equal to 12.5 or it corrodes steel (Carbon steel SAE 1020) at a rate greater than 0.250 inches per year at a test temperature of 54 °C.

Solid waste is considered reactive if, upon mixing with water, it exhibits instability, violent reactions, and the generation of toxic vapours. It is also reactive if it contains cyanide or sulfide and, when exposed to acidic conditions, produces toxic vapours. Additionally, it is reactive if it detonates or explodes when exposed to standard temperatures, heat, or pressure.

Solid waste is considered toxic if, using the leaching procedure, test method 1311 (EPA), the extract from a representative sample of the waste contains metallic pollutants (arsenic, barium, cadmium, chromium, silver, lead, and selenium) and organic contaminants (1,1-dichloroethylene, tetrachloroethylene, trichloroethylene, carbon tetrachloride, chlordane, endrin, lindane, heptachlor, heptachlor epoxide) at a concentration equal to or exceeding the allowed value specified by the United States Code of Federal Regulations 40 CFR 261.

In Europe, the ecotoxicological assessment of solid waste is a regulatory requirement. Council Directive 91/689/EEC establishes 14 criteria for the characterization of hazardous waste. Criterion H 14 "Ecotoxic" in Annex III considers hazardous waste "substances and preparations that present or may present immediate or delayed risks to one or more sectors of the environment".

This assessment is carried out through ecotoxicity tests that measure, under laboratory conditions, the impact of human-origin contaminants on terrestrial and aquatic ecosystems [52] and aim to determine the dose-response relationship, indicating the concentration at which the contaminant causes harm to organisms. The extent of these damages depends on the type of test and the characteristics of the bioindicator organism used [52]. To ensure reproducibility and comparability of results, it is important to employ standardized protocols. Therefore, the UNE (Spanish Association for Standardization) has standardized the UNE 14735 guide for the preparation of waste samples intended for ecotoxicity testing. The ecotoxicity tests established by the UNE-EN 14735 standard are internationally harmonized by CEN, ISO, or OECD, meeting criteria of ecological relevance, and sensitivity to toxins, resulting in reproducibility, viability, reliability, and robustness [53].

Earthworms were used for the ecotoxicological analysis as they are the most abundant soil-dwelling invertebrates [54], and are considered "ecological engineers" and key indicators of the health of this environmental compartment [55]. Despite their importance in ecosystem balance and environmental monitoring, there are few studies on the toxicity of dyes to these organisms [56]. The studies developed have mainly focused on the toxicity of azo dyes [56,57]. Among the earthworms, *Eisenia fetida*, also known as the Californian red earthworm, is considered by ISO and OECD as a model species in the study of soils contaminated by xenobiotics [58,59]. According to previous studies, the optimal moisture level in organic substrates for their growth and reproduction is 85% of dry mass, otherwise, they could dehydrate and die [60]. Their thin cuticle makes them susceptible to changes in their environment, and they can accumulate contaminants that cause adverse effects on their growth and reproduction [54]. It has been described that microorganisms present in the gut of earthworms degrade organic matter into small particles that serve as nutrients for the worms, and these, in turn, promote further microbial activity through their faecal matter [61].

The present study aimed to evaluate the hazardousness and ecotoxicity of paiche leather residues dyed with natural and synthetic dyes to contribute to establishing competitive advantages of implementing clean technologies in the technological development of paiche leather tanning. For this purpose, (i) the dyeing active compounds of natural dyes extracted from turmeric and purple corn and synthetic acid dyes applied in the dyeing of paiche leather were characterized, (ii) the quality of the leather obtained with both types of dyes were evaluated by determining their fastness properties, (iii) the hazardousness of untreated and dyed leather residues with synthetic and natural dyes was assessed by determining their flammability, corrosivity, reactivity and toxicity, (iv) the ecotoxicity of leather residues was evaluated using the bioindicator organism Eisenia fetida. Thus, this research contributes to generating knowledge regarding the tanning of paiche leather, with an emphasis on dyeing, as there are few studies on this type of leather. Furthermore, it contributes to understanding the impact on soil ecosystems due to the presence of residues containing azoic acid dyes that have no usage restriction.

EXPERIMENTAL

Materials

The paiche skins were obtained in the Pucallpa region, Peru, with Certificate of Origin No. 167-2021 issued by the Regional Government of Ucayali. Turmeric roots and purple-corn cobs were sourced from local suppliers in the city of Lima.

Dyeing process of paiche leather

Both turmeric roots and corn cobs were dried and ground to a particle size of 120 mesh. The dyeing compounds, curcumin and anthocyanins, respectively, were extracted in an aqueous medium, following the detailed procedure outlined in the flowchart shown in Figure 1.

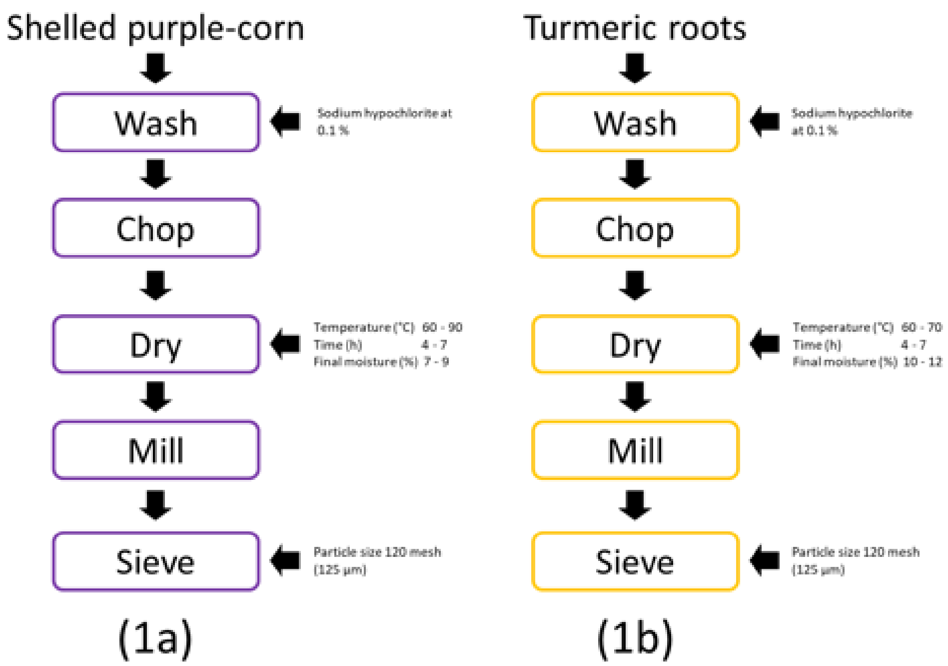

Figure 1. Extraction process of dyeing active compounds. (1a) Extraction process from purple corn. (1b) Extraction process from turmeric roots

After completing the extraction, the identification of the dyeing compounds was carried out using UV-visible spectrophotometry. An Agilent spectrophotometer, model Cary 60 UV-VIS, with Cary WinUV software, was employed in the range of 200-600 nm for turmeric, 400-700 nm for purple corn and 400-

700 for the azoic acid dyes. The readings of the samples were conducted at the Chemistry Laboratory of CITEccal Lima.

The paiche skins intended for dyeing were tanned with synthetic phenolic syntans (7.5%) and glutaraldehyde (2.5%). The dyeing process, both with natural and synthetic dyes, was carried out according to the detailed instructions in Table 1. The synthetic dyes used were azoic acid dyes from the CODYECO brand, Ambranile LGN yellow, and Ambranile 54B violet. In the process, a fatliquoring agent (Fosfoliker 61.46L), naphthalene-sulfonic acid (RO-EXP), and aniline fixative (TOP AGENT A) and formic acid were used. It is worth noting that in this research, no finish was applied to the dyed skins. The dyeing process was coded as paiche leather without dyeing (PL.WD), paiche leather dyed with purple-corn dye (PL.PC), paiche leather died with turmeric dye (PL.CL), paiche leather dyed with synthetic dyes violet (PL.SP) and yellow (PL.SY).

Table 1. Formulations used in the dyeing and fatliquoring processes of paiche leather

Test	Stage	Product	Amount (%w/w)	Units	Time	Control
PL.PC	Dyeing	Water (45 °C)	200.0%	L	60 min	45 °C
		Purple-corn colourant	10.0%	kg		
		Alum	17.0%	kg		
		Salt	10.0%	kg		
	Fatliquoring	Fatliquoring agent	2.0%	kg	40 min	Room temperature
PL.CL	Dyeing	Water (45 °C)	200.0%	L	60 min	45 °C
		Turmeric colourant	5.0%	kg		
		Alum	15.0%	kg		
	Fatliquoring	Fatliquoring agent	2.0%	kg	40 min	Room temperature
PL.SP	Dyeing	Water (40 °C)	30.0%	L	60 min	40 °C
		Naphthalene-sulfonic acid	1.0%	kg		cutting and piercing
		Violet powder aniline	2.0%	kg		
	Fatliquoring	Water (60 °C)	100.0%	L	60 min	60 °C
		Fatliquoring agent	10.0%	kg		
		Fungicide	0.15%	kg	45 min	Once ended, drain
		Formic acid	1.0%	kg		
	Enhancement	Water (50 °C)	100.0%	L	10 min	50 °C
		Violet powder aniline	1.0%	kg		
		Aniline fixative	0.4%	kg	20 min	
		Formic acid	2.0%	kg	10 min	
PL.SY	Dyeing	Water (40 °C)	30.0%	L	60 min	40 °C
		Naphthalene-sulfonic acid	1.0%	kg		cutting and piercing
		Yellow powder aniline	2.0%	kg		
	Fatliquoring	Water (60 °C)	100.0%	L	60 min	60 °C
		Fatliquoring agent	10.0%	kg		

Test	Stage	Product	Amount (%w/w)	Units	Time	Control
PL.SY	Fatliquoring	Fungicide	0.15%	kg	45 min	Once ended, drain
		Formic acid	1.0%	kg		
	Enhancement	Water (50 °C)	100.0%	L	10 min	50 °C
		Yellow powder aniline	1.0%	kg	10 min	
		Aniline fixative	0.4%	kg	20 min	
		Formic acid	2.0%	kg	10 min	

Note: All percentages of chemicals for dyeing and fatliquoring were calculated based on the shaved weight

The dyeing of tanned paiche skins was carried out in a twin-load stainless steel drum, 7WAY brand model GHE II R801-2. Two tanned skins were dyed for each type of dye. The dyeing process took place at the Tanning Pilot Plant of CITEccal Lima.

The physical-mechanical properties of the leather regarding colour fastness were measured according to the method “NTP ISO 11640:2014, 'LEATHER - Colour fastness tests - Colour fastness to and fro rubbing'.”, the results for leather dyed with natural extracts (PL.PC, PL.CL) were compared to those established by standards and those obtained by leather died with synthetic dyes (PL.SY, PL.SP).

Preparation and characterization of samples for hazardousness and ecotoxicity analyses

The preparation of the samples was carried out following the procedures established in the UNE-EN 14735 and NTP-ISO 4044 standards. Each of the tanned and dyed skins was cut into strips and chopped using electric scissors and a chopper, reduced to particles of approximately 4 mm x 4 mm with a MUVER 5003 mill. Subsequently, the samples were stored in hermetically sealed bags.

In the leather samples, pH measurement was conducted in accordance with the NTP-ISO 4045 standard. For this procedure, a WTW pH meter, INOLab Multi 9020 model, a mechanical stirrer from MUVER, 5070 series, a water purification system from HIDROMAQ, MAQ100RUV-P model, and an analytical balance from AND, GR 300 model, were employed. Merck buffers 7 and 4 were used to calibrate the equipment. The determination of dry matter content and humidity percentage was carried out using the gravimetric method, employing a precision balance from AXIS (+/- 0.01 grams), AKA4200G model, and an oven from BINDER, FD 115 model. These characterizations were performed at the Chemistry and R&D Laboratories of CITEccal Lima, respectively.

The hazard analysis was conducted on samples of paiche leather dyed with turmeric and synthetic yellow dye, following the standard methods established in the United States Federal Regulation Code, 40 CFR 261. The parameters assessed are described in Table 2. This analysis was carried out at the LABECCO SRL laboratory.

Table 2. Parameters evaluated to determine the hazardous characteristics of paiche leather.

Evaluation according to the U.S. Federal Regulation Code 40 CFR 261		
Parameter	Method	Criteria
Ignitability	EPA 1030 Rev. 1:2014	The leather sample is placed on a refractory material in the form of a 200 mm strip to be subjected to the heat of a 1000 °C flame (ignition source). The sample exhibits ignitability characteristics if: (1) The application of the flame (ignition source) is maintained until ignition occurs and the flame runs in the strip of material or for a maximum time of 2 minutes. (2) It is observed if the combustion spreads along the 200 mm of the strip of material during 4 minutes. If steps (1) and (2) are not fulfilled, the test is terminated.
Reactivity - H_2S	EPA 9034 Rev 0:1996	The sample exhibits reactivity characteristics if, when exposed to pH conditions between 2.0 and 12.5, it can generate toxic gases, vapours or fumes in quantities of 250 mg HCN/kg of residue or 500 mg H2S/kg of residue.
Reactivity - HCN	EPA 9014 Rev 1:2014	
Corrosivity	EPA 1110A Rev 1:2004	To determine if the waste has hazardous characteristics, it must be determined if the corrosion rate is less or greater than 6.35 mm per year. In the case of being greater than 6.35, it will be a waste that possesses this characteristic.
Metals	EPA 1311 Rev 0:1992	For a waste to be considered toxic it must exceed the maximum concentration of at least one of the toxic constituents indicated in the method.
Volatile Organic Compounds	EPA 8260 D Rev 4:2017	
Organophosphorus Pesticides	EPA 8270 D. Rev 5:2014	
Organochlorine Pesticides	EPA 8081 B. Rev 2:2007	

The content of metals and hexavalent chromium was determined in samples of undyed leather to establish a baseline level of this metal before the dyeing process. The methodologies used for the determination of hexavalent chromium were EPA 3060 and EPA 7196 A. This analysis was conducted at the LABECCO SRL laboratory.

Ecotoxicological assessment using the California red worm (*Eisenia fetida*)

The ecotoxicological assessment was based on the UNE-EN ISO 11268-1 standard, which establishes mortality as the test parameter to determine the ecotoxicological effect of contaminants on earthworms (*Eisenia fetida*), also known as red Californian earthworms.

The earthworms were obtained from the National Agrarian University La Molina, selecting individuals that met sexual maturity requirements (presence of clitellum), were over 3 months old, and weighed between 300 and 600 mg. Before being placed in the test containers, the earthworms were acclimatized to the substrate and provided with a diet of decomposing fruits and vegetables for one week.

As a substrate, gardening soil was used, acquired from the Las Flores market located in the Rímac district in the city of Lima, Peru. The pH and conductivity of the substrate were measured in a soil:water suspension at a 1:1 ratio. The soil texture class was determined using the hydrometer method, and the organic matter content was evaluated using the Walkley and Black method. These measurements were carried out in the Soil, Plants, Water, and Fertilizers Analysis Laboratory at La Molina Agrarian University. On the other hand, the dry matter content and percentage of humidity were determined according to the UNE-ISO 11465 standard in the R&D Laboratory of CITEccal Lima. Figure 2 shows pictures of the *Eisenia* and the leather waste substrates used for the assessment.

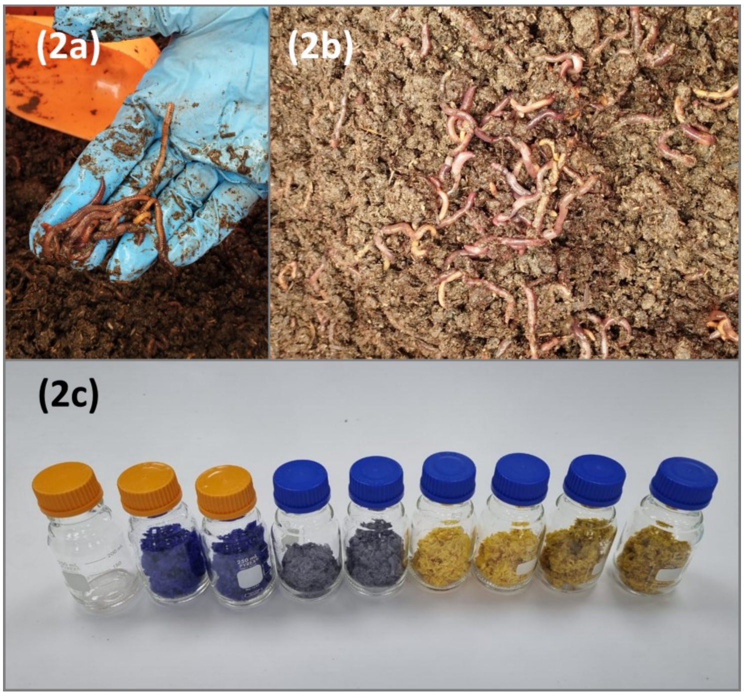

Figure 2. Ecotoxicological assessment pictures of species and substrates. (2a) and (2b) *Eisenia fetida* earthworms used in ecotoxicity tests. (2b) Leather samples prepared for pH determination, in duplicate. From left to right, samples of leather dyed with synthetic purple dye, leather dyed with purple corn, leather dyed with synthetic yellow dye, leather dyed with turmeric

In the experimental design, each ground leather sample was mixed with the substrate to obtain the following concentrations: 0.02, 0.04, 0.08, 0.16, and 0.20 g leather/g substrate. For each treatment, 20 sexually mature earthworms were selected and placed in the test containers. The design concentrations were established based on the tolerance concentration for wet blue leather shavings of bovine origin, as determined by Buleje and Marrufo [62], which is equal to 0.04 g leather/g substrate after 12 weeks. Each treatment had 4 replicates. As a negative control (0.00 g leather/g substrate), substrate without added leather was used, and the same procedure as in the treatments was followed [62].

Once the preparation stage was completed, the containers were placed in a ventilated environment without direct exposure to the sun and protected from dust. The moisture level in the medium was controlled, maintaining a constant level of 70% [63]. The count of dead earthworms was conducted weekly over thirteen weeks. The percentage of mortality was calculated using Equation 1.

$$\%MP = \frac{LE_i + LE_f}{LEi} * 100\% \quad (1)$$

Where:

%MP = *Mortality Percentage*

LEi = *Initial Live Earthworms*

LEf = *Final Live Earthworms*

To determine the acute toxicity of the leather samples on earthworms, the median lethal concentration (LC50) was determined after two weeks or 14 days. Chronic toxicity was assessed based on LC50 after thirteen weeks of exposure. Californian earthworms are considered to have a life cycle of around 4 months [64,65].

Statistical Analysis of Data

In the statistical analysis of the ecotoxicological experimental design, the Kruskal-Wallis test was employed to assess significant differences between treatments at a significance level of 0.05 [66]. To identify significant differences between pairs of treatments, the Dunn-Bonferroni test was used [67,68]. The Real STATISTICS Excel add-in was utilized for the statistical analysis. The LC50 was determined through Probit analysis [69–71].

RESULTS AND DISCUSSION

Spectrophotometric characterization of natural dyes

Figure 3 presents the UV absorption spectrum for the dye extracts of purple corn and turmeric in an aqueous medium. In Figure (3a), the absorption peak of anthocyanins at 515 nm is observed, which is the main dyeing principle of purple corn. Saha et al. indicate that the maximum absorption (λ_{max}) in the visible region is generally recorded around 510 - 520 nm for anthocyanins. In Figure (3b), the absorption peak of curcumin at 425 nm is shown, which is the main dyeing principle of turmeric [72]. Figure (3c) shows the synthetic yellow dye characterized by maximum absorption at 441 nm [73], and in Figure (3d), the synthetic purple dye is characterized by maximum absorption at 549 nm, both attributed to the chromophores containing azo linkage [74,75].

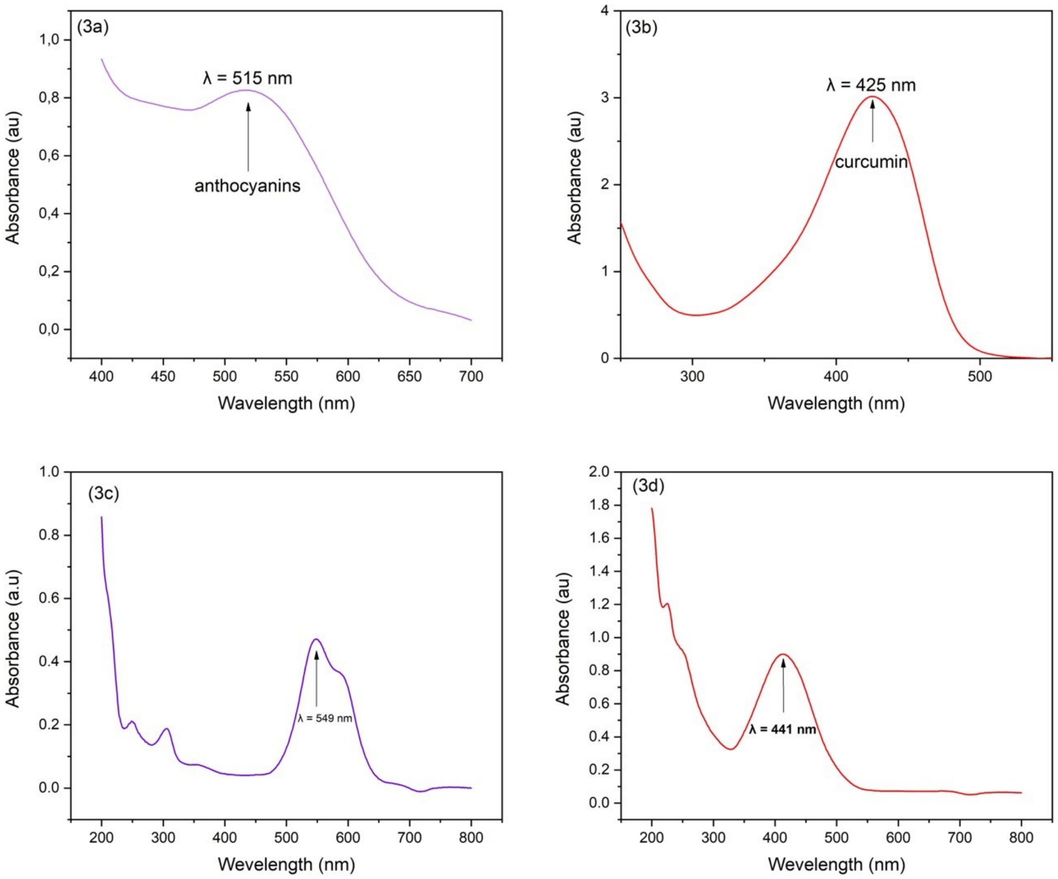

Figure 3. The absorption spectrum of dye extracted in aqueous solution: (3a) Spectrum from purple-corn dye, (3b) Spectrum from turmeric dye, (3c) Spectrum for de synthetic purple dye, (3d) Spectrum from synthetic yellow dye

Characterization of leather and substrate samples

Table 3 displays the results obtained for the fastness tests, and Figure 4 displays the samples of leather dyed in the present study. The obtained results were compared with proposed requirements for fancy and fine leathers, which include exotic leather. Additionally, they were compared with proposed specifications for clothing and leather goods. In regards to dry rubbing colour fastness, when a change of colour was evaluated, it was observed that all samples, whether dyed with synthetic or natural dye, met the requirement of 3 or higher. In the results of wet rubbing colour fastness, the minimum value obtained was 4. Regarding resistance to artificial sweat, all samples received a rating of 4/5.

However, when staining was evaluated, in wet rubbing colour fastness, samples dyed with synthetic dyes obtained a rating of 2/3, while natural samples achieved a minimum score of 3.

Regarding resistance to sweat, the values for samples dyed with synthetic dyes received low ratings of 2 and 1/2, whereas natural samples scored 3 and 3/4. The low ratings in samples dyed with synthetic dyes could be attributed to their formation of ionic bonds with the leather, resulting in low water and perspiration resistance, unlike other dyes such as reactive dyes that form covalent bonds, which would enhance their solidity [4,76].

Concerning lightfastness, the results from Table 3 show that synthetic dyes exhibit greater resistance to light [4,76]. It has been determined that the photostability of synthetic dyes may depend, among other factors, on their chemical structure, which determines the reactivity of excited states formed by the action of light, varying according to the attached functional groups, in this case, to the azo groups [77]. The identification of attached functional groups constitutes a methodological limitation of the present research and represents an aspect to be investigated concerning its toxicity.

In the case of natural colourants, their exposure to ultraviolet light in the presence of air could lead to oxidation, where oxygen free radicals or superoxide ions may discolour the dye [78]. Improved photostability could be achieved by incorporating UV absorbers or antioxidants. This aspect presents a relevant area for further investigation [78].

The skin of the paiche fish presents a unique appearance, determined by the imprint left by the scales when removed. It is not a smooth surface, as it features thin, overlapping laminae resembling roof tiles, which previously housed the scales. It is important to highlight that by not using chemical dehairing substances in the process and applying a white tanning agent, the texture and colour of the skin remain intact, including the characteristic dorsal spot of the species. Although this area may appear slightly darkened after dyeing, it represents an intrinsic and distinctive characteristic of the leather. Nonetheless, the colour in the dyed leather is uniform and has penetrated the entire structure, from the grain side to the flesh side. As a final result, the softness of the leather is also particular, yet suitable for the crafting of clothing and leather goods. Establishing parameters for the characterization and standardization of this exotic skin is another area of future research and development.

Table 3. Results of colour fastness for leather samples

Leather type or application	Dry rubbing colour fastness (grain side) 50 cycles	Wet rubbing colour fastness (grain side) 20 cycles	Artificial sweat solution colour fastness (grain side) 20 cycles	colour Lightfastness[3]
Fancy and fine leathers[1]	≥ 3	≥ 3	≥ 3	≥ 3 is taken as a reference value (72 hours, exposure to yellow light)

Leather type or application	Dry rubbing colour fastness (grain side) 50 cycles	Wet rubbing colour fastness (grain side) 20 cycles	Artificial sweat solution colour fastness (grain side) 20 cycles	colour Lightfastness[3]
Clothing[2]	≥ 3	≥ 3	≥ 3	≥ 3 is taken as a reference value (72 hours, exposure to yellow light)
Leather goods[1]	≥ 3	≥ 3	≥ 3	
PL.SY	3 (change of colour) 3/4 (staining)	4/5 (change of colour) 2/3 (staining)	4/5 (change of colour) 2 (staining)	5
PL.SP	4 (change of colour) 3 (staining)	4 (change of colour) 2/3 (staining)	4/5 (change of colour) 1/2(staining)	4/5
PL.CL	5 (change of colour) 4(staining)	4/5 (change of colour) 3/4 (staining)	4/5 (change of colour) 3/4(staining)	2/3
PL.PC	4 (change of colour) 4/5 (staining)	4 (change of colour) 3 (staining)	4/5 (change of colour) 3 (staining)	3/4

[1] Gerhard J. Possible defects in leather production.

[2] NTP ISO 14931:2016. LEATHER: Guide for the selection of leather for clothing (excluding fur skins).

[3] Evaluated using a grayscale scale. Maximum evaluation score equal to 5.

The results of colour fastness obtained for the natural dyes are comparable to those obtained by other studies on other types of leather. Bai studied the dyeing of bovine leather with turmeric [79]. The fastness data they reported ranged between 1/2 for lightfastness, 4/5 for dry rubbing fastness, and 3/5 for wet rubbing fastness. They also evaluated the leather's fastness to dry cleaning, obtaining a rating of 4 for colour change. The use of mordants with aluminium, copper, and iron salts did not modify these results. Bai evaluated the effect of Chestnut's inner skin as a mordant on cow leather dyed with turmeric powder [80]. The mordanting was evaluated both as pre and post, determining that the former increased lightfastness from 1-2 to 2/3. Regarding dry rubbing fastness (4/5) wet rubbing fastness (3/4), and colour change fastness to washing (4), no variations were observed. Asma Inat et al. dyed goat leather with turmeric, evaluating oxalic acid, acetic acid, and citric acid as mordants. They found that mordanting with oxalic acid increased both dry and wet rubbing fastness, as well as staining and colour change, to a rating of 5. The results of the evaluation without mordanting were 3 (dry) and 3/4 (wet) for staining, and 3/4 (dry) and 4 (wet) for colour change [21,79,80].

While no prior records were found for the use of purple corn in leather dyeing, upon reviewing the application of anthocyanins extracted from other plant species, such as *Garcinia mangostana Linn* peels, ratings of 4/5 were found for dry rub fastness at 150 cycles and for wet rub fastness at 50 cycles in colour change [24].

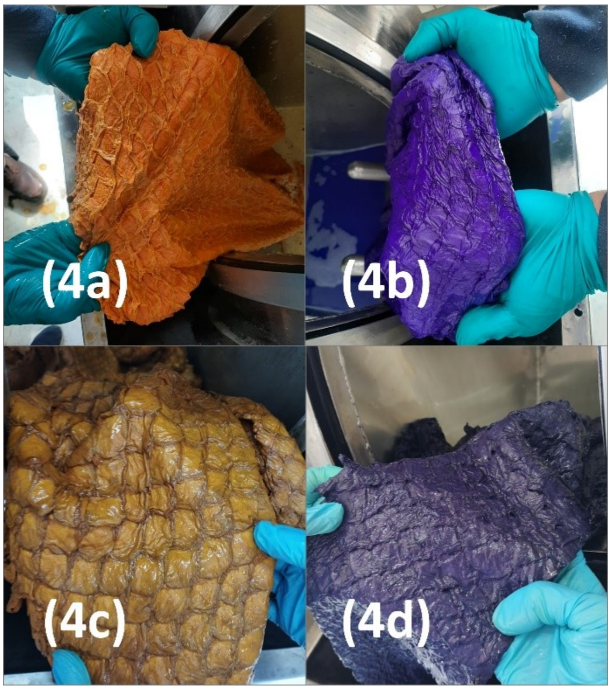

Figure 4. Samples of leather dyed. (4a) shows the sample PL.SY. (4b) shows the sample PL.SP. (4c) shows the sample PL.CL. (4d) shows the sample PL.PC

Regarding the other parameters characterized, the pH results in the leather samples are presented in Table 4, where it can be observed that the purple-corn dyed paiche leather (PL.PC) and the paiche leather without dyeing (PL.WD) have a pH below 7, while the turmeric-dyed (PL.CL) and synthetic-dyed leathers, purple (PL.SP) and yellow (PL.SY), are in a range between 7.3 and 7.9.

The recommended pH for leather, according to technical standards NTP 241.023, NTP 241.021, and NTP 241.022 for casual footwear, men's footwear, and women's footwear, respectively, is set at a value greater than or equal to 3.5. Therefore, the dyed samples meet this requirement.

As for the moisture percentage in the leather, it ranges from 8.25% to 12.12%, a range considered suitable for preserving the physico-mechanical properties of the leather, such as flexibility and softness [81].

The pH value obtained for the substrate was 7.92, falling within acceptable values for the survival of the red Californian earthworm. According to García-Sánchez et al. and Wu et al., these worms can survive in a pH range from 5 to 9, with optimal conditions being those close to neutrality [82,83].

The moisture content was determined to be 33.79%, so once the substrate was mixed with the residues, it was necessary to add distilled water to maintain it around 70%. This process was carried out every two weeks. It has been observed that the growth and reproduction of earthworms can occur in a moisture range of 55% to 85% [60]. Additionally, studies have concluded that with 85% humidity, cocoon production and fertility are significantly higher [60].

The result of electrical conductivity (E.C) of the substrate, which was 5.58 dS/m, was found below the threshold necessary for the survival of earthworms, set at 8 dS/m. According to García-Sanchez et al., values above this threshold could lead to earthworm mortality [82].

The soil texture was identified as sandy loam, with a composition of 71% sand, 19% silt, and 10% clay. Previous studies have indicated that sandy soils tend to decompose organic additions more rapidly compared to clayey soils [84]. Additionally, it has been reported that soil texture favours reproduction over mortality [60].

On the other hand, it has been concluded that soil suitable for the development of *Eisenia fetida* earthworms must have a high pore volume, good moisture absorption capacity, high water retention capacity, optimal aeration, and structural stability against decay [60]. The presence of organic matter has a significant influence on preserving these soil properties. In this study, an organic matter content of 16.40% was recorded, which is considered suitable for the optimal development of earthworms [60].

Table 4. Results of pH measurement, dry matter content, and moisture in samples of paiche leather without dyeing, paiche leather dyed with natural dyes, and synthetic dyes

Sample	pH	Dry matter content (%)	Moisture (%)
PL.WD	5.86	11.52	13.03
PL.PC	4.15	8.71	9.54
PL.CL	7.90	7.62	8.25
PL.SP	7.30	10.81	12.12
PL.SY	7.50	9.94	11.04

Results of the Hazardousness Analysis

Table 5 presents the results of flammability, reactivity, and corrosivity. The flammability results, with values below 2.2 mm/s, indicate a low capacity to sustain the spread of fire for both materials. During the experiment, it was observed that applying an ignition source to PL.CL and PL.SY caused momentary ignition. However, upon extinguishing the ignition source, the fire quickly went out.

The reactivity analysis indicated that the samples do not contain cyanides and sulfides in their composition. When exposed to pH conditions between 2.0 and 12.5, no gases, vapours, or toxic fumes were generated in quantities equal to or greater than 250 mg of HCN/kg or 500 mg of H2S/kg.

Regarding the corrosivity analysis, since the samples are solid, pH was used as the evaluation criterion, which is detailed in Table 4. According to the results, none of the leather samples have a pH less than or equal to 2 or greater than or equal to 12.5, indicating that they lack corrosive characteristics.

The values recorded for the content of metals and organic compounds in Table 5 are below the permissible limits in the United States Federal Regulation Code, 40 CFR 261. This suggests that there is no significant toxicity evident.

On the other hand, in Table 6, a comparison is presented between the levels of heavy metals in PL.WD, PL.CL and PL.SY, concerning the metal limits set in COMMISSION DECISION (EU) 2016/1349, which establishes ecological criteria for granting the EU Ecolabel for footwear. It is observed that all three leather samples exceed the established chromium limit. Nonetheless, leather without dyeing shows the lowest chromium concentration, while the sample dyed with synthetic dye exhibits the highest amount. The presence of chromium in the leather samples could originate due to cross-contamination caused by residual traces of chromium present in the tanning drums, coming from previous tanning processes [85]. However, it is also possible that the chromium content comes from residues present in the chemical products, including dye substances.

Since the total chromium values exceeded the permitted limits in all leather samples, a specific analysis of hexavalent chromium (Cr^{6+}) was conducted on the undyed leather. The obtained result, which was below the detection limit set at 0.1 mg/kg, also fell below the limit established by the EU. This outcome is plausible, considering that traces of total chromium could originate from the basic chromium sulfate tanning process, where chromium is in the III oxidation state. This finding suggests that there are no oxidizing conditions leading to the formation of Cr^{6+} [52,86,87].

Another metal that exceeded the limit established in the EU ecological label for footwear is nickel, which can be part of the composition of stainless steel [88]. Since the tanning dyeing drums are made of steel, the presence of nickel in the samples could be attributed to its migration during the dyeing processes.

Additionally, it is observed that samples of paiche leather dyed with turmeric or aniline exceed the limit values for lead and cadmium according to regulations. The presence of these metals could originate from the chemicals used in tanning and dyeing processes, where metals might be present as traces. Furthermore, the dyes could contribute to this presence, as they could come from the use of fertilizers or as a result of environmental contamination [89]. These results demonstrate the necessity of considering product life cycle analysis from raw material extraction when scaling up this type of technology.

Table 5. Hazardous parameters result in flammability, reactivity to H_2S, reactivity to HCN, heavy metals, and organic compounds. Parameters were evaluated on PL.CL and PL.SY, compared with values established by the United States Federal Regulation Code 40 CFR 261

Hazard Test		Units	Limit of Quantification (LoQ)	Permissible Level	PL.SY	PL.CL
Ignitability		mm/s	2.2	2.2	<LoQ	<LoQ
Reactivity - H_2S		mg H2S/Kg	0.010000	250	<LoQ	<LoQ
Reactivity - HCN		mg HCN/Kg	0.010000	500	<LoQ	<LoQ
Inorganic toxicity	Arsenic	mg As/L	0.003000	5.0	<LoQ	<LoQ
	Barium	mg Ba/L	0.003000	100.0	0.3220	0.2620
	Cadmium	mg Cd/L	0.000200	1.0	<LoQ	<LoQ
	Chromium	mg Cr/L	0.000400	5.0	0.4708	0.4088
	Silver	mg Ag/L	0.001000	5.0	<LoQ	0.0020
	Lead	mg Pb/L	0.006000	5.0	0.0110	0.0110
	Selenium	mg Se/L	0.007000	1.0	<LoQ	<LoQ
Organic toxicity	1,1-Dichloroethene	mg/L	0.000530	0.7000	<LoQ	<LoQ
	Tetrachloroethene	mg/L	0.000620	0.7000	<LoQ	<LoQ
	Trichloroethene	mg/L	0.000850	0.5000	<LoQ	<LoQ
	Carbon tetrachloride	mg/L	0.000750	0.5000	<LoQ	<LoQ
	Chlordane	mg/L	0.000004	0.0300	<LoQ	<LoQ
	Endrin	mg/L	0.000002	0.0200	<LoQ	<LoQ
	Lindane	mg/L	0.000006	0.4000	<LoQ	<LoQ
	Heptachlor	mg/L	0.000004	0.0080	<LoQ	<LoQ
	Heptachlor Epoxy	mg/L	0.000004	0.0080	<LoQ	<LoQ

Table 6. Metals concentration measured in PL.WD, PL.CL and PL.SY in comparison with limits established by COMMISSION DECISION (EU) 2016/1349

Parameter	Units	Limit of Detection (LoD)	Permissible Level	Limit 1	Limit 2	PL.WD	PL.SY	PL.CL
Antimony	mg Sb/Kg	0.60	-	30.00	30.00	<0.60	<0.60	<0.60
Arsenic	mg As/Kg	1.40	5.00	0.20	1.00	<1.40	<1.40	<1.40
Cadmium	mg Cd/Kg	0.02	1.00	0.10	0.10	0.36	<0.02	<0.02
Cobalt	mg Co/Kg	0.06	-	1.00	4.00	<0.06	<0.06	<0.06
Copper	mg Cu/Kg	0.09	-	25.00	50.00	11.39	3.09	4.08
Chromium	mg Cr/Kg	0.08	5.00	1.00	2.00	8.67	22.57	18.56
Nickel	mg Ni/Kg	0.15	-	1.00	1.00	15.36	1.05	4.92
Lead	mg Pb/Kg	0.18	5.00	0.20	1.00	<0.18	0.55	0.49

Results of the ecotoxicity analysis

The survival count of earthworms in the acute toxicity assessment for the negative controls (Figures 5, 6, and 7) indicates that there were no significant differences between the counts of weeks 1 and 2, with the initial quantity of earthworms remaining constant.

Regarding chronic toxicity, it is observed that in the negative control of the undyed leather trial, according to the data in Figure 5, a 50.0 % mortality rate is reached by week 13. In the negative control of the trial with leather dyed with purple corn, a 12.5 % mortality rate was recorded by week 13, while in the negative control of the trial with leather dyed with turmeric, a 10.0 % mortality rate was observed by week 13. The difference between these last two controls and the undyed leather could be determined by the characteristics of the substrate, mainly its homogeneity.

Nevertheless, overall, the substrate appears to be a suitable habitat for the earthworms. However, over the weeks, the depletion of organic matter and, consequently, the food source for the earthworms could begin to cause mortality in the individuals [90,91]. It has been reported that the percentage of organic carbon in the substrate decreases up to 105 days with *E. fetida* [65]. It should be noted that the presence of other chemical substances in leather may contribute to the toxicity of the substrate. For example, traces of formaldehyde from the synthesis of the tanning agents used in the tanning could be present. Formaldehyde has been classified as a carcinogenic and teratogenic substance by the World Health Organization (WHO), and the European Chemicals Agency (ECHA) has prioritized it as one of the Substances of Very High Concern (SVHC) [92].

Figures 5, 6, and 7 present the results of the survivor count for the different treatments, including PL.WD, PL.PC, and PL.CL respectively. Each treatment included a negative control and the concentrations 0.02, 0.04, 0.08, 0.16, and 0.20 g leather/g substrate (w/w) are coded in the figures as C02, C04, C08, C16 and C20 respectively.

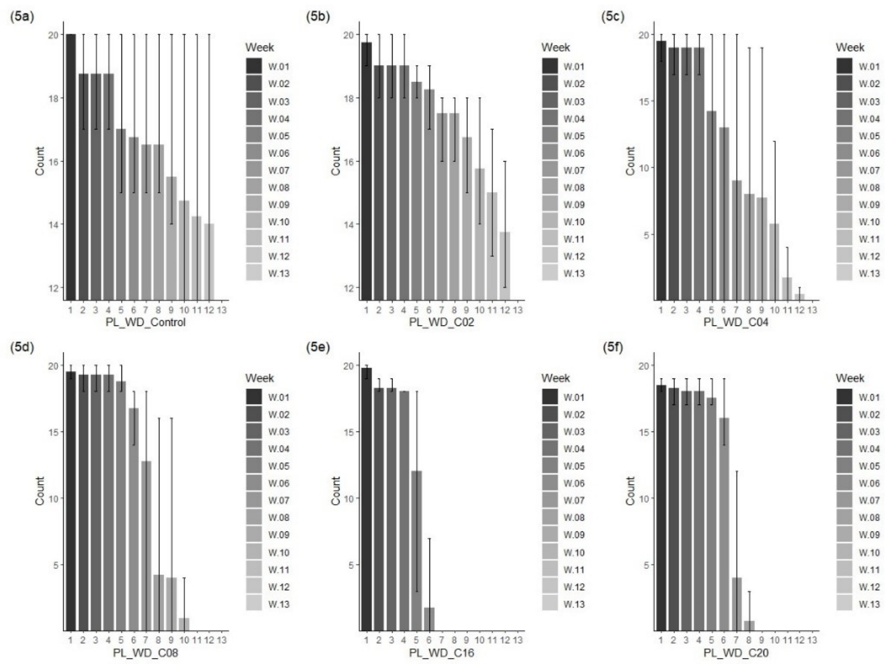

Figure 5. Count of surviving worms in the toxicity of PL.WD for 13 weeks: (4a) PL.WD negative control (w/w), (4b) PL.WD at 0.02 (w/w), (4c) PL.WD at 0.04 (w/w), (4d) PL.WD at 0.08 (w/w), (4e) PL.WD at 0.16 (w/w), (4f) PL.WD at 0.20 (w/w)

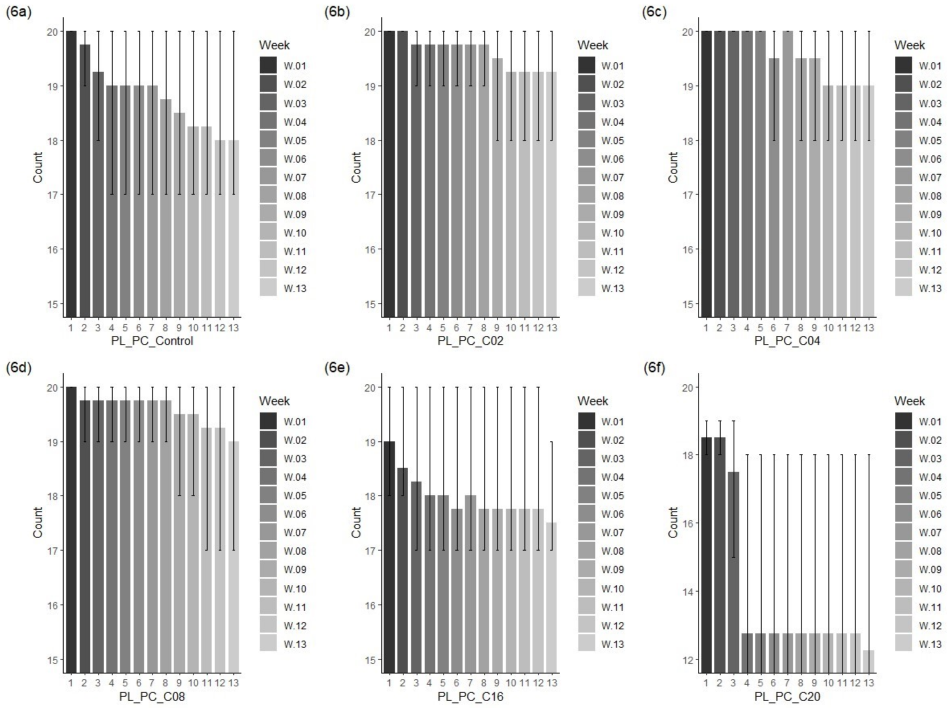

Figure 6. Count of surviving worms in the toxicity of PL.PC for 13 weeks, (5a) PL.PC negative control (w/w), (5b) PL.PC at 0.02 (w/w), (5c) PL.PC at 0.04 (w/w), (5d) PL.PC at 0.08 (w/w), (5e) PL.PC at 0.16 (w/w), (5f) PL.PC at 0.20 (w/w)

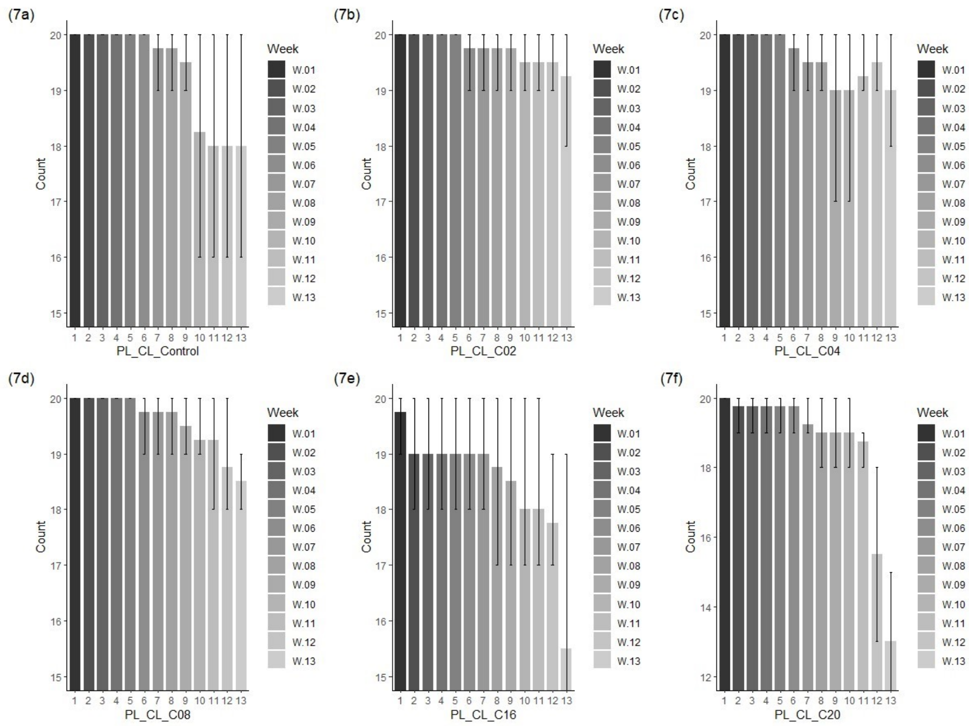

Figure 7. Count of surviving worms in the toxicity of PL.CL for 13 weeks: (6a) PL.CL negative control (w/w), (6b) PL.CL at 0.02 (w/w), (6c) PL.CL at 0.06 (w/w), (6d) PL.CL at 0.08 (w/w), (6e) PL.CL at 0.16 (w/w), (6f) PL.CL at 0.20 (w/w)

Figure 8 presents the comparison of acute toxicity among the studied leathers. In particular, the mortality of the earthworms exposed to undyed leather after 14 days is 7.5 % in the treatment with 0.2 g leather/g substrate. This value is comparable to the mortality obtained for the treatment with 0.2 g leather/g substrate of leather dyed with purple corn, which is equal to 7.0 %. This suggests that the dyeing process does not increase the toxicity of the leather. On the other hand, in the treatment with leather dyed with purple aniline, 100 % mortality is reached in the treatment with 0.04 g leather/g substrate, demonstrating higher toxicity.

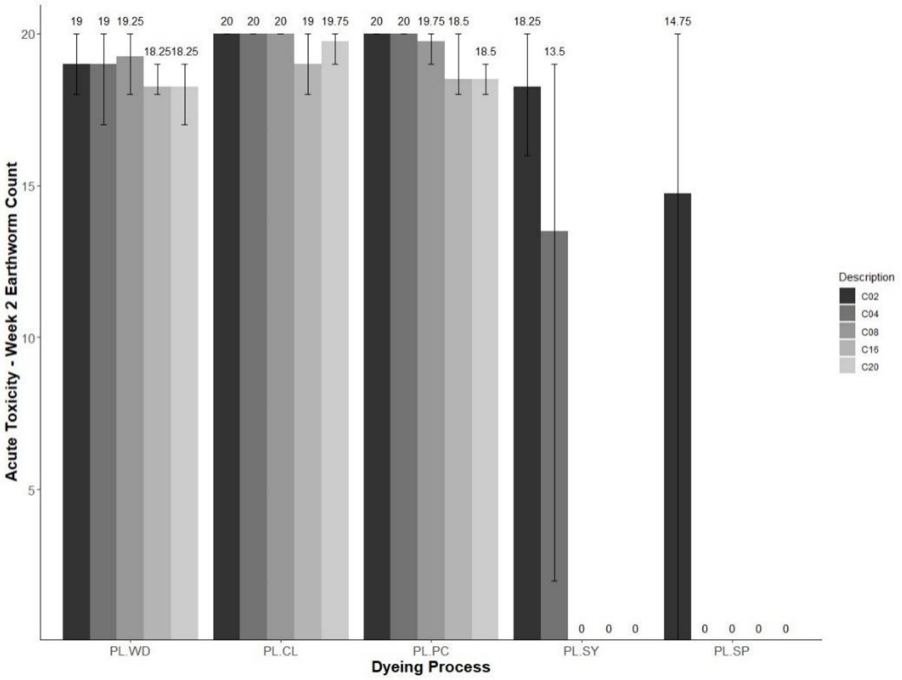

Figure 8. Acute toxicity comparison, expressed as earthworm counted after 14 days of exposure for PL.WD, PL.CL, PL.PC, PL.SY and PL.SP

In the case of leather dyed with turmeric, in the treatment with 0.2 g leather/g substrate, 100% survival is recorded at 14 days. Regarding the PL.SY, in the treatment with 0.04 g leather/g substrate, a mortality of 17.5% is recorded, and in the treatment with 0.08 g leather/g substrate, a mortality of 100% is reached. This demonstrates high toxicity compared to turmeric (PL.CL).

This result suggests that the incorporated active compounds, mainly curcumin, could provide nutrients that enhance the survival of earthworms compared to undyed leather. Furthermore, the toxicity of synthetic dyes may be attributed to the toxic action of their molecular structure or the degradation products generated by microorganisms present in the soil or associated with earthworms. To determine this mechanism, it is necessary to complement this research by identifying, the type of aromatic amines and/or other degradation products produced. Comparing the mortality rate of earthworms in residues of leather dyed with turmeric to the ones placed in residues of leather dyed with purple corn using the Kruskal-Wallis Test, it can be observed that there is no significant difference between the effects of these dyes on the earthworms mortality rate. These results would further support the conclusion that the active compounds of natural dye substances and their degradation products are likely to be more biodegradable and less toxic than synthetic ones.

In the analysis of the chronic toxicity of PL.WD, carried out by the probit method, an LC50 equal to 0.035 g leather/g substrate is obtained for week 8. In the treatment with 0.02 g leather/g substrate, 32.5% is reached in week 12. Therefore, leather tanned with phenolic compounds and glutaraldehyde presents higher toxicity compared to wet blue leather shavings, of bovine origin, tanned with basic chromium sulfate. For the latter, a tolerable concentration (NOEC) equal to 0.04 g leather/g substrate was determined in week 12 [62].

The difference could be explained in terms of the type of tanning agent used, expecting higher toxicity in chromium-tanned leather [93]. However, this will depend on the degradation products present, the bioavailability of substances in the residues, and the conditions of the substrate. Therefore, further tests under similar conditions are required to establish significant differences, while also identifying possible traces of formaldehyde associated with the tanning agents [94].

The Kruskall-Wallis statistical analysis indicates that there are significant differences, with a confidence level of 95 %, between the treatments of the negative control and the treatment with 0.02 g leather/g substrate of undyed leather starting from week 5. This suggests that from that week onwards, certain levels of toxicity from undyed leather are beginning to be identified. Regarding chronic toxicity, results expressed in Figure 9 allow the comparison between the different samples at week 13. For the PL.PC, a mortality rate of 42.5 % is reached in the 0.2 g leather/g substrate treatment in week 13, and for PL.CL, in the same week, a mortality rate of 40 % is achieved for the same treatment.

An important aspect to highlight is the observation of high rates of cocoon production in the experiments, up to the eighth week in PL.WD, up to the eleventh week in PL.PC, and up to the thirteenth week in PL.CL. Since the reproduction rate largely depends on the quality of food and substrate [61], it can be asserted that the residues of leather dyed with natural dyes and the substrate form a suitable mixture for the development of earthworms.

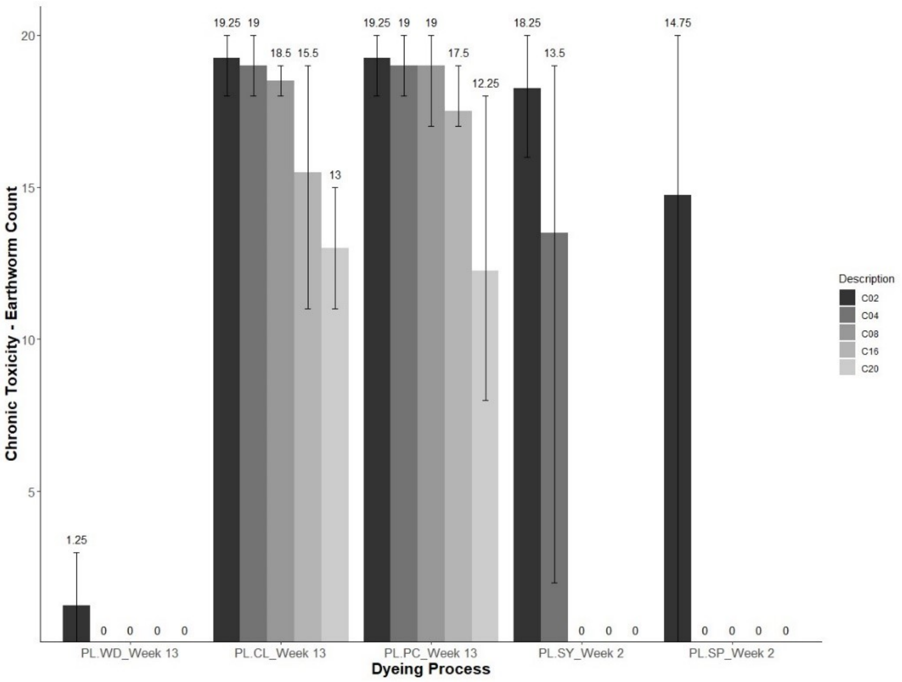

Figure 9. Chronic toxicity comparison, expressed as earthworm counted after 13 weeks of exposure for PL.WD, PL.CL, PL.PC, PL.SY and PL.SP

The lower toxicity of natural dyes can be attributed to the properties of their active compounds. For example, in the case of turmeric, curcumin, the main colouring agent, has demonstrated health benefits for humans, including anti-atherogenic, anti-inflammatory, antioxidant, and anticarcinogenic activity [95–97]. Požgajová et al. reported that mice treated with curcumin were widely protected against the adverse effects of cadmium in terms of lipid and protein peroxidation [98]. Curcumin has also been shown to have a protective effect against persistent organic compounds such as acetonitrile, polychlorinated biphenyls, dioxins, Bisphenol A (BPA), and phthalates [99]. Furthermore, in plant species, the same protective effect against the action of the herbicide pendimethalin has been demonstrated through the *Allium cepa L.* assay, evaluating cytological, biochemical, and physiological parameters [100].

In the case of purple corn, several studies demonstrate a positive interaction between *Eisenia fetida* and *Zea mays L*. For instance, Zhang, Z. et al., reported on a study where fertilizers were prepared from *Eisenia fetida* mucus, showing that it favoured the increase of nutrients in the substrate, improved physiological parameters of maize, promoted its growth, and enhanced dry matter accumulation. Lemtiri et al., in an experiment with *Eisenia fetida* and *Zea mays L.*, reported that they modified the availability of certain heavy metals, such as cadmium, influencing plant health [101]. Canellas et al. found that *Eisenia fetida* worms produce humic substances that influence the promotion of *Zea mays L.* growth. Chen et al. demonstrated that during the vermicomposting process, the worms promoted the decomposition of corn stalks, based on the analysis of vermicompost microbial communities through high-throughput pyrosequencing [102,103].

It is worth noting that the difference in pH between leather dyed with purple corn and turmeric extracts is likely to not affect the earthworms. This is because the mixture of leather with the substrate would fall within the suitable pH range for the development of earthworms.

According to the EU Ecolabel regulations, the only restricted azo dye is Acid Red 26. However, similar to the findings of this research, other studies have reported direct toxicity of dyes and/or their metabolites during degradation when exposed to bioindicator organisms.

In the exposure of *Allium cepa* to Acid Orange 7 (AO7), an azo dye commonly used in the tanning industry, presumably non-toxic and unregulated, negative effects were reported on root growth, shoot biomass, chromosomal anomalies, and reduction of the mitotic index [11]. Similarly, exposure of the freshwater fish *Labeo rohita* to this type of dye caused sublethal effects such as changes in swimming pattern and balance, mucus secretion, alterations in gill histology, haemorrhages, and changes in liver and kidney morphology [104]. On the other hand, exposure to the fish *Anabas testudineus* evidenced a decrease in enzymatic activity in the liver, kidney, and muscle tissues directly correlated with the increase of reactive oxygen species (ROS), as well as alterations in the normal architecture of liver tissues such as degenerated and necrotic hepatocytes, and absence of nucleus [105].

Other azo dyes, such as Yellow 5, Yellow 6, Red 2, and Red 40, exposed to zebrafish embryos, caused alterations in behaviour patterns such as hatching difficulty and developmental abnormalities, including cardiac oedema, decreased heart rate, yolk sac oedema, and spinal defects such as spinal curvature and tail distortion [7].

On the other hand, microbial degradation of azo dyes Yellow 17, Violet 7, and Orange 52 by *Pseudomonas putida* resulted in metabolites with genotoxic effects. Conversely, it was demonstrated that the dye Acid Black 210 (AB210), degraded by the bacterium *Providencia sp.*, produced metabolites such as benzene, naphthalene, and 4-aminophenyl-N-(4-aminophenyl) benzene sulfonamide, which reduced the dye's toxicity in microbial toxicity tests and in the A. cepa roots comet assay [106]. Regarding soil bioindicator organisms, there were no records found of *Eisenia fetida* exposure to acid dyes. However, a study on the exposure to synthetic solutions of azo dye Direct Black 38 (DB38) and copper phthalocyanine dye Reactive Blue 15 (RB15) [56] on different trophic levels reported swelling and the bottleneck effect in *Eisenia fetida*.

CONCLUSION

The results of dry, wet, and artificial sweat rub fastness obtained for leather dyed with natural colourants are comparable to those for leather dyed with synthetic colourants, meeting the standards for footwear and leather goods manufacturing. This demonstrates that the substitution of synthetic colourants is technically feasible. However, the lightfastness properties should be strengthened through the study of UV absorbers or antioxidants, preferably of natural organic type, to increase their fastness and contribute to obtaining more sustainable products

The hazard analysis results indicated that these samples are not flammable, corrosive, or reactive to hydrogen sulfide and cyanide. Furthermore, they do not exhibit toxicity due to heavy metals or organic compounds, in accordance with EU regulations. However, the presence of traces of chromium, nickel, lead, and cadmium, in undyed leather, leather dyed with turmeric and leather dyed acid dye, exceeds the permissible limits for footwear. So there is a need to study the product's life cycle analysis, with emphasis on the origin of raw materials and inputs, as well as the process conditions, including equipment.

Regarding the ecotoxicity of leather residues, assessed using earthworms as bioindicators, it is concluded that leathers dyed with colourants extracted from purple corn and turmeric do not exhibit significantly different acute toxicity compared to leather without dyeing. The acute toxicity of leather dyed with purple acid dye, expressed as mortality, was 100.0 % at a concentration of 0.04 g leather/g substrate. Additionally, the toxicity of leather dyed with yellow acid dye reached approximately 100.0 % mortality at a concentration of 0.08 g leather/g substrate. Conversely, the survival rate of earthworms in treatments with leather dyed with turmeric and purple corn was 100%. These results indicate that the acute toxicity of leather dyed with natural dyes is significantly lower compared to leather dyed with synthetic dyes.

In terms of chronic toxicity, an LC50 equal to 0.035 g leather/g substrate was determined in week 12 for paiche leather without dyeing. For leather dyed with purple corn, a concentration of 0.2 g leather/g substrate resulted in 42.5% mortality in week 13, while for leather dyed with turmeric, mortality reached 40% in the same week at the same concentration. These results suggest that extracts from purple corn and turmeric reduce the chronic toxicity of leather without dyeing, thanks to the beneficial properties of their active ingredients.

The results of this research demonstrate that the toxicity of the studied acid dyes for soil quality bioindicator organisms is relevant and concerning. Therefore, it is necessary to delve into the degradation mechanisms of these dyes and study other dyes, including acids, used in the leather industry that have not been restricted by regulations. Similarly, it is necessary to expand studies on ecotoxicity to other bioindicator organisms of aquatic ecosystems, as the release of these dyes also occurs in wastewater. This involves evaluating the effectiveness of treatment systems in removing these contaminants and, consequently, mitigating the toxicity of these chemical substances.

Author Contributions

Conceptualization and methodology – Marrufo-Saldaña L, Paucar-Palomino M; formal analysis – Marrufo-Saldaña L, Paucar-Palomino M, Barra-Hinojosa J, Requena-Sánchez N; writing-original draft preparation – Marrufo-Saldaña L, Paucar-Palomino M, Barra-Hinojosa J, Requena-Sánchez N; writing-review and editing – Marrufo-Saldaña L, Paucar-Palomino M, Barra-Hinojosa J, Requena-Sánchez N; supervision – Marrufo-Saldaña L. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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