

# DAMAGE OF PICKLED HIDES, WET-BLUE LEATHER AND VEGETABLE TANNED LEATHER DUE TO BIODETERIORATION

by

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## ABSTRACT

Fungi and bacteria can be responsible for undesirable changes in hides and leather. This study identifies some of the defects caused by fungal growth on pickled hides, wet blue leather and vegetable-tanned leather, such as stains, protein material loss, deterioration of grain layer, and modification of the physical and mechanical properties of resistance. The assessment of the samples exposed to microbiological attack was carried out through visual observation, scanning electron microscopy (SEM), tensile strength test and determination of mass loss. Leather without preservation or treated with insufficient of antimicrobial agent to prevent fungal contamination showed changes in the structure, loss of protein material, a reduction in physical and mechanical properties as well as the presence of stains that may compromise the quality of the final product.

## INTRODUCTION

A great concern in the leather industry is the deterioration of hides and leather due to the development of microorganisms. Biodeterioration is an important factor impairing aesthetic, functional and other properties of leather and the products made from them. Besides having high moisture, the hides and leather are rich in fats, proteins and carbohydrates that serve as substrates for the growth of microorganisms, especially bacteria and fungi. These microorganisms produce enzymes able to degrade macromolecules to smaller units that can be absorbed through the cell membranes and be used as nutrients and energy source; they are capable of infecting the hides from slaughter to the already processed leather.<sup>1,2,3</sup> Some species of bacteria and fungi synthesize important substances of this substrate, which can lead to a variety of defects as a result of microbial activity.

After skinning, raw hides are attacked by bacteria, which can lead to its complete decomposition by microbial enzymes,

which can only be interrupted by the use of preservatives. As the hides are processed (chrome tanning, vegetable-tanning, fat liquoring, drying, finished leather) the bacteria give way to another group of microorganisms, the fungi.

When bacteria affect the hides it begins the process of putrefaction, the release of odors and hairslip. Once this stage is reached, shortly after, the bacteria begin to attack the fibers of the grain layer, causing tiny abrasions known as 'pin holes'. Furthermore, damage to the epidermal layer results in a matt and lusterless grain, and loss of the skin substance can also occur.<sup>4,5</sup>

The loss of skin substance due to putrefaction process originates empty or spongy leather with reduced resistance. In addition to these damages, it may also happen the development of colored stains (red due to the development of halophilic bacteria and extremely halophilic archaea), demarcation of blood vessels (veininess) in the grain side, as well as stains due to the release of fatty acids.<sup>1,6,7</sup>

The predominant bacteria found in raw hides (green hides and salted hides) and soaking baths are *Enterobacter aerogenes*, *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Aerobacter* and *Micrococcus*.<sup>8,9,10</sup>

In the previous study, Orlita<sup>1</sup> verified that the growth of halophilic bacteria on salted hides results in production of a range of pigments giving, especially, red and violet spots. From the colored spots *Micrococcus roseus*, *M. luteus* and *M. morrhuae* have most frequently been isolated. In another study, researchers observed grain side severely disturbed, as hair shafts are lying on the surface and reveals the disruption of the fiber structure in the hide samples that showed growth of halophilic microorganism *Haloferax gibbonsii*.<sup>11</sup>

The degree of damage in leather produced from hide samples inoculated with halophile bacteria showed strong temperature dependence, according to Bailey and Birbir. In these

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experiments, within seven weeks, no damage was observed on brine-cured hide samples held at 4°C, however at a temperature of 40°C was observed damage the grain on hide samples caused by extremely halophile organisms.<sup>7</sup>

Fungi can attack salted hides, pickled hides, vegetable tanned leather, chrome tanned leather and finished leathers. Semi-processed leathers like wet blue and wet white are extremely sensitive to fungal attack due to their high water content and acidity. Fungal growth on salted hides may cause severe damage such as looseness or loss of grain layer, weak fiber, weak grain, pitted grain and stains.<sup>12,13</sup> In pickled hides or after the tanning, the growth of fungi hinders a uniform dyeing, causing differences in shades of color of the finished leather. Some parts may show matting with a blemished appearance. The fatliquoring may present difficulties due to lack of uniformity in the absorption of oils, which is processed differently in areas affected by fungi.

The growth of fungi on leathers can lead to a variety of undesirable defects such as pigmented spots, which are difficult to remove, non-uniform dyeing and finishing, reduction of physical and mechanical properties and harmful changes in the leather surface. These damages affect the quality of the final product and reduce the leather's value or the value of the products manufactured with it. In addition to financial losses, there are also indirect losses to rework and customer dissatisfaction.<sup>14,15</sup>

In the leather industry, the main fungi that attack the leather are: *Aspergillus niger*, *Penicillium chrysogenum*, *Aspergillus flavus*, *Rhizopus nigricans*, *Mucor mucedo*, *Trichoderma sp.*, *Penicillium herguei*.<sup>1,2,16</sup>

Fungi such as *Paecilomyces ehrlichii* (*Penicillium klebanii*), *P. aculeatum*, *P. purpurogenum* and *P. roseopurpureum* were thought to be producers of the red spots on chrome tanned leather. During drying of leathers the growth of fungi such as *A. ochraceus*, *A. wentii*, *P. rugulosum*, *P. funiculosum*, *P. variotii* and *V. glaucum* may cause spots of various sizes in green, yellow-brown, dark-brown, grey and brown-green shades.<sup>1</sup>

Bitlisli and collaborators<sup>13</sup> reported that the growth of fungi such as *Aspergillus terreus*, *A. niger*, *A. fumigatus*, *Penicillium restrictum*, *P. citrinum*, *Alternaria sp.* and *Cladosporium sp.* in hides salted affect the fixing of dyestuff in the leather as well as homogeneity of the dyeing.

In the leather industry the correct use of antimicrobial agent is necessary for the conservation of hides and leather to ensure the quality of the final product. Thus, this study aims to evidence the importance of using antimicrobial agents to avoid the growth of fungi which cause loss of protein substance, appearance of pigmented stains, microstructural and physical-mechanical damage on pickled hides, wet-blue leather and vegetable tanned leather.

## MATERIALS AND METHODS

The microbiological test of biodegradation in the soil was performed in leather tanned with chromium and leather tanned with vegetable tannin, with and without the addition of antimicrobial agent, in order to check the mass loss (protein material). Also, tensile strength assays were performed (tension and elongation at break rupture) after exposure to fungal attack with *Aspergillus niger* in tropical chamber to evaluate impairment of physical and mechanical properties. In addition, samples of pickled hides, wet-blue and vegetable-tanned leather, were subjected to plating test, which was carried out in the presence of contaminant fungi *Aspergillus niger*, *Aspergillus flavus* and *Penicillium herguei*, were assessed with the naked eye and by scanning electron microscopy to examine the appearance of the grain side, stains on the leather and biodeterioration caused by fungal attack.

The tests were performed using a pickled hide (pre-acidified hide came from a tannery) of 22 kg cut on pieces of approximately 40 g. The control samples (1) were prepared without antimicrobial agent treatment. The hides were preserved during the pickle or the tanning with the antimicrobial agent (2) 2-thiocyanomethylthio benzothiazole (TCMTB); (3) Isothiazolin; (4) Oil dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol - 2-ylcarbamate (OIT+BMC/oil); (5) Aqueous dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-N-benzimidazol - 2-ylcarbamate (OIT+BMC/water); and (6) 2-n-octyl-4-isothiazolin-3-one (OIT).

### Sample Preparation

In the following described different treatments the quantities of chemicals were calculated in percentage of mass of the hide pieces for the pickle or tanning processing carried out in rotating laboratory drums.

1) Pickle: pieces of hide were treated with 200% water, 0.2% formic acid, 0.2% sulfuric acid for 30 minute. Then 0.2% antimicrobial agent was added for processing during 180 minutes.

2) Chromium tanning: pieces of hide were treated with 200% water, 0.2% formic acid and 0.2% sulfuric acid for 30 minutes; followed by addition of 9% basic chromium salt to continue processing during 120 minutes; after this 0.2% antimicrobial agent was added for processing more 180 minutes. Then 0.5% magnesium oxide was added for the chromium fixation during 360 minutes, where the final pH had to be between 3.8 – 4.2.

3) Vegetable tanning: pieces of hide were treated with 200% water, 0.2% formic acid and 0.2% sulfuric acid for 30 minutes; 1.2% sodium sulfate for 120 minutes, after the bath was drained off. New tanning bath was carried with 100% water and 30% vegetable tanning (mimosa extract), added in three parts in time intervals of one hour, and after the last addition

of vegetable tanning were soaking for more 120 minutes. The fatliquoring was carried out in the same tanning bath with more 50% water, 7% sulfited oil, 6% sulfated oil and 0.2% antimicrobial agent, rotating in the drum for one hour, then more 100% water and 1% formic acid, helped the oil and antimicrobial agent distribution and fixation for 20 minutes.

Hide samples were treated following the same procedures (pickling, chromium tanning and vegetable tanning) but at no stage was added to the antimicrobial agent. These samples were used as control (sample without antimicrobial agent).

#### Biodeterioration Process in Soil

Heterotrophic microorganisms (fungi and bacteria), which are responsible for microbial deterioration, also play a role in the decomposition of organic compounds, turning it into a source of carbon and energy. This results in loss in total mass, and causes changes in the initial physical and chemical composition of the decomposed material.

In this assay, six samples of wet-blue and vegetable-tanned leathers (dimensions: 7.0 cm × 3.0 cm), one control and five samples, were treated with different antimicrobial agent. These samples were buried in organic fertilized soil at a depth of 10 cm. The mass loss of the buried samples (dry weight) was determined after 45 days.

#### Leather Tensile Strength Test

The maximum tensile strength and elongation at break of leather was measured to assess loss of resistance of the leather fibers due to fungal attack. Samples of both wet-blue and vegetable-tanned leather were analyzed in a tropical chamber. The samples were incubated in this chamber under conditions specified by the American Society for Testing and Materials (ASTM) D7584-10 standard test.<sup>17</sup> The samples were maintained at a controlled temperature of 30°C, under highly humid conditions, and subjected to an atmosphere containing fungal (Petri dish containing *Aspergillus niger* was incubated with the samples and placed at the bottom of the tropical chamber) for 28 days.

Tests to determine tensile strength and elongation at break tests were conducted according to the NBR 11.041 standard test<sup>18</sup>, using the universal testing machine AME-5kN (Volumec, Farra di Soligo, Italy). This machine determines the maximum strength required to tear leather, and simultaneously assesses the elongation at break.

#### Stains and Damage Caused by Fungal Attack

Samples of pickled hide, wet-blue, and vegetable-tanned leather were plated according to the ASTM D4576-08 standard test<sup>19</sup>. The plates containing the leather and hide samples, culture media, and fungal spores were incubated at a temperature of 28°C for 28 days. The samples were then washed to remove fungi adhered to the surface and to evaluate the stains and damage caused by biodeterioration.

**Examination of the Infected Pickled Hides, Wet-blue Leather and Vegetable Tanned Leather Samples by SEM**  
Morphological modifications of the leather sample surface due to deterioration of the leather caused by *Aspergillus niger*, *Aspergillus flavus* and *Penicillium hergueli*, were evaluated through scanning electron microscopy (SEM).

## RESULTS AND DISCUSSION

#### Biodeterioration Process in Soil

Table I shows the percentage of loss in mass of the wet-blue and vegetable-tanned leather samples caused by microbial biodeterioration in soil, 45 days after treatment with antimicrobial agent.

Major losses in mass were noticed in the vegetable-tanned leathers, and chiefly occurred in the control vegetable leather, which was not treated with antimicrobial agent. This suggests the display of a higher sensitivity towards microbial attacks by untreated leather. This effect may also be due to the presence of nutrients such as simple sugars, which are present in the plant extracts and oils that are added during lubrication in the tanning process, and which may serve as food for the microorganisms.

#### Leather Tensile Strength Test

Tables II and III display the results of tensile strength evaluation in chrome and vegetable-tanned leather samples that were incubated in a tropical chamber and the ones that were contaminated by *A. niger* have asterisks. Regarding the leather wet-blue only the control samples were superficially contaminated by *A. niger* fungi. On the other hand all

**TABLE I**  
**Mass loss of samples undergoing biodeterioration in soil.**

Antimicrobial agent	Mass loss (%)	
	Wet-blue leather	Vegetable-tanned leather
Control	14,33	22,65
TCMTB	6,82	14,29
Isothiazolin	8,47	10,98
OIT+BMC/oil	7,71	14,44
OIT+BMC/water	3,73	17,02
OIT	6,34	14,93

vegetable-tanned leather samples submitted to assay in tropical chamber showed fungal growth. To facilitate better comparison of the contaminated and uncontaminated vegetable-tanned leather one sample of vegetable-tanned leather not incubated in the tropical chamber (uncontaminated vegetable-tanned leather) was also evaluated.

Chrome tanned leather samples displayed a higher tensile strength and elongation at break, compared to the vegetable-tanned leather. Impaired resistance was observed in the control sample of wet-blue leather contaminated with *A. niger*. The tensile strength and elongation at break values for the control were lower than those presented by antimicrobial agent treated wet-blue leather. This suggests that poor preservation and fungal contamination in leathers leads to structural changes and alterations in their physical-mechanical properties.

Differences between the control (displaying fungal contamination) and antimicrobial agent treated-samples were less pronounced in vegetable-tanned leathers, as all five types of antimicrobial agent were found to be ineffective (offers of 0.2%), and as such, unable to prevent attack by *A. niger*. The uncontaminated sample that was not attacked by the fungus displayed high tensile strength and elongation at break.

#### Stains and Damage Caused by the Fungal Attack

The Figures 1 and 2 show samples of wet-blue leather and vegetable-tanned leather contaminated with fungi.

Figures 3 - 8 show the stains and deterioration of pickled hide, wet-blue leather, and vegetable-tanned leather samples which were contaminated with *Aspergillus niger* and *Penicillium herguiei*.

By visual inspection it was found that samples where fungal growth occurred showed patches of various hues (red, green and black) after washing. These stains are believed to be a result of the action of different genera of fungi that produce color pigments characteristic of the fungus, specific to the substrate (pickled hide, wet-blue and vegetable leather). The removal of these stains is difficult, or even impossible, and often compromises the quality of the final product. Indeed, it could hinder the subsequent steps in the leather making process, and cause problems such as for non-uniform dyeing, or even prevent the production of white or light colored leathers. In addition, the loss of protein material was also observed in fungus-infected pickled hide samples (Figure 4).

Images of the samples obtained by scanning electron microscopy (Figures 9, 10 and 11) revealed a remarkable change in the leather layer structure in the contaminated samples as compared to the uncontaminated samples. These images showed that the uncontaminated samples presented smooth and intact surfaces, with well-defined pores, and no fiber damage. On the other hand, contaminated

samples displayed deteriorated surfaces and irreparable damage (rough surfaces and damaged fibers) in response to the microbial attack. Fungi utilize the components in hide and leather for nutritional purposes.

**TABLE II**  
**Tensile strength of wet-blue and vegetable-tanned leather.**

Antimicrobial agent	Tensile strength (N/mm <sup>2</sup> )	
	Wet-blue leather	Vegetable tanned leather
Control	11,39*	7,16*
TCMTB	14,55	7,45*
Isothiazolin	14,87	8,01*
OIT+BMC/oil	13,71	9,61*
OIT+BMC/water	17,23	9,20*
OIT	16,48	9,73*
Uncontaminated vegetable-tanned leather		11,37

\*Samples contaminated by fungi in the tropical chamber assay.

**TABLE III**  
**Elongation at break in wet-blue and vegetable-tanned leather.**

Antimicrobial agent	Elongation at break (%)	
	Wet-blue leather	Vegetable tanned leather
Control	70,74*	55,50*
TCMTB	84,51	36,65*
Isothiazolin	86,84	57,19*
OIT+BMC/oil	75,18	56,51*
OIT+BMC/water	81,84	40,17*
OIT	91,84	59,47*
Uncontaminated vegetable-tanned leather		60,51

\*Samples contaminated by fungi in the tropical chamber assay.

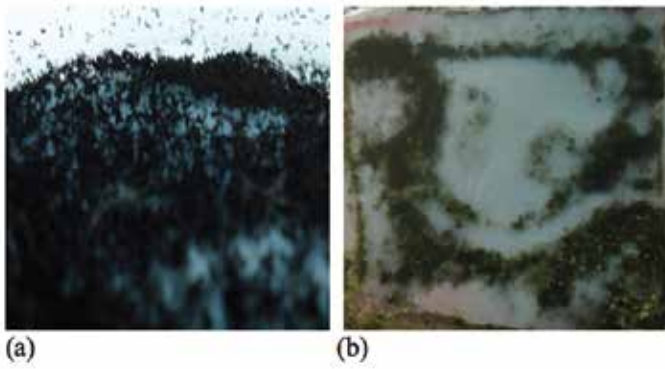


Figure 1. Wet-blue leather contaminated with (a) *Aspergillus niger* and (b) *Penicillium herguei*.

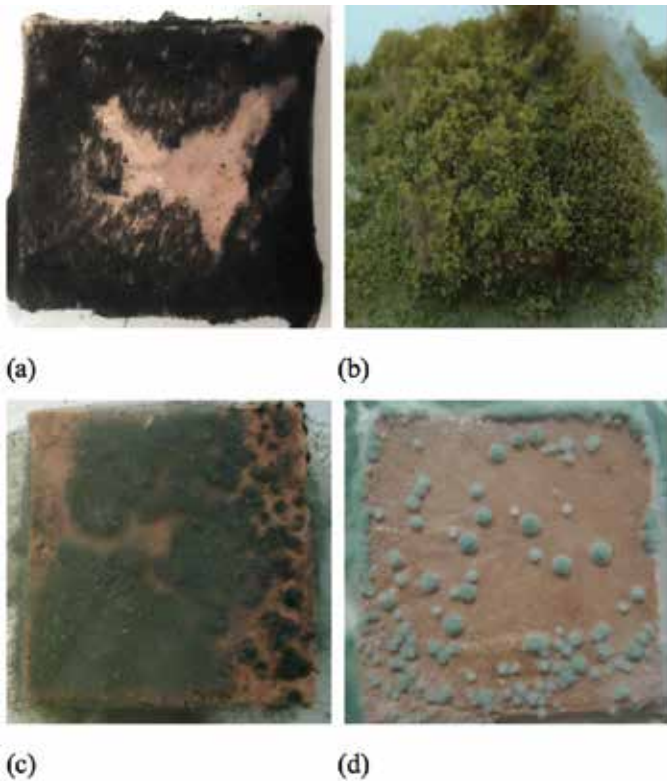


Figure 2. Vegetable-tanned leather contaminated with (a) *Aspergillus niger*, (b) *Aspergillus flavus*, (c) *Penicillium herguei* and (d) *Penicillium chrysogenum*.



Figure 3. Stains and degradation of pickled hide samples degraded by *Penicillium herguei*.



Figure 4. Degradation of pickled hide samples caused by *Aspergillus niger*.

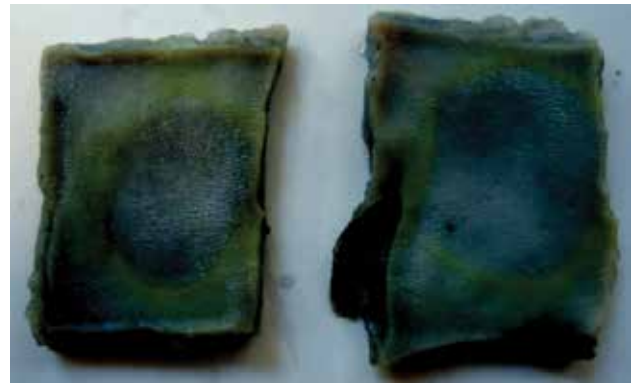


Figure 5. Stain on the wet-blue leather samples caused by *Aspergillus niger*.

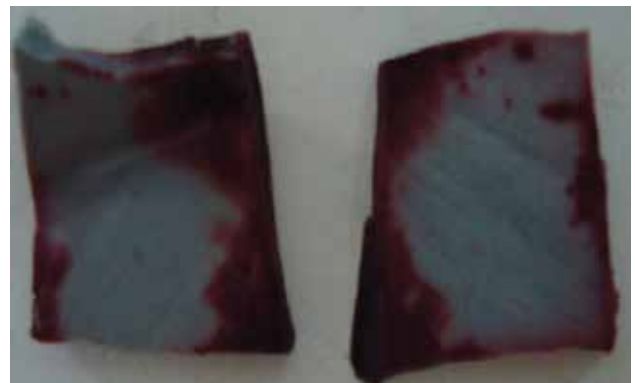


Figure 6. Stain on the wet-blue leather samples caused by *Penicillium herguei*.

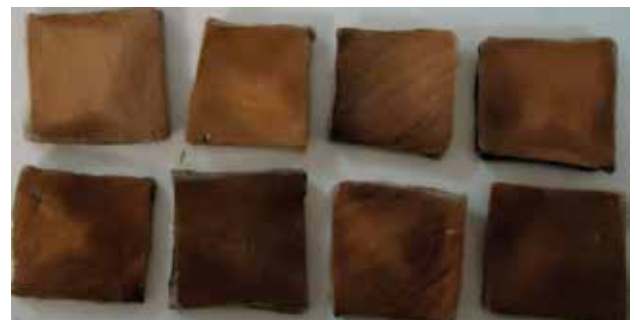


Figure 7. Stain on the vegetable-tanned leather samples caused by *Aspergillus niger*.

CONCLUSIONS

The appropriate use of antimicrobial agent is essential for the protection hides and leather, and is therefore of utmost importance to the leather industry. In the process of biodegradation in soil a decrease in mass of the samples due to fungal attack, showing the structures of a modification of leather with loss of protein material. The results of tensile test showed that hides without preservation or poorly preserved (insufficient concentration of antimicrobial agent to prevent fungal contamination) and suffered a loss on physical-mechanical properties. The reduction of physical and mechanical properties observed in wet-blue leather and vegetable tanning, which suffered fungal contamination, can be explained by the significant mass reduction (protein material) and the damage to the leather surface observed by scanning electron microscopy. Stains caused by fungal contamination, which were observed on the leather surfaces, may compromise the quality of the final product.



Figure 8. Stain on the vegetable-tanned leather samples caused by *Penicillium herguei*.

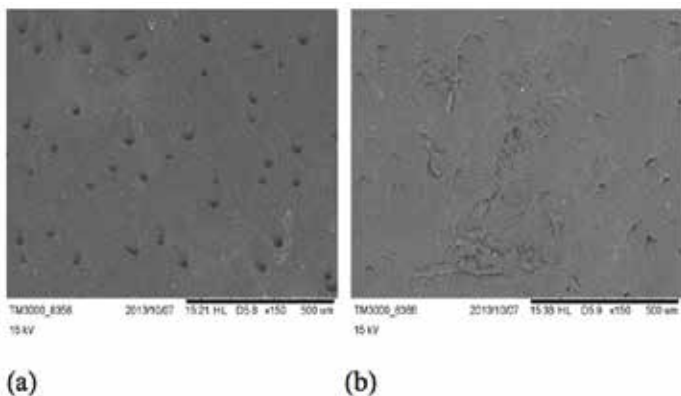


Figure 9. Pickled hide samples (a) uncontaminated and (b) contaminated with *Aspergillus niger*.

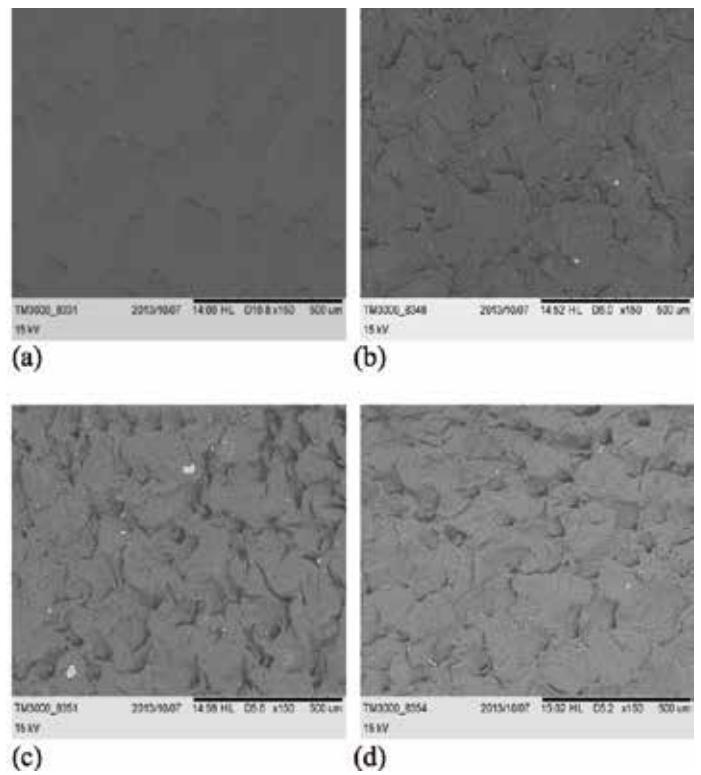


Figure 10. Wet-blue leather samples (a) uncontaminated, (b) contaminated with *Aspergillus niger*, (c) *A. flavus*, and (d) *Penicillium herguei*.

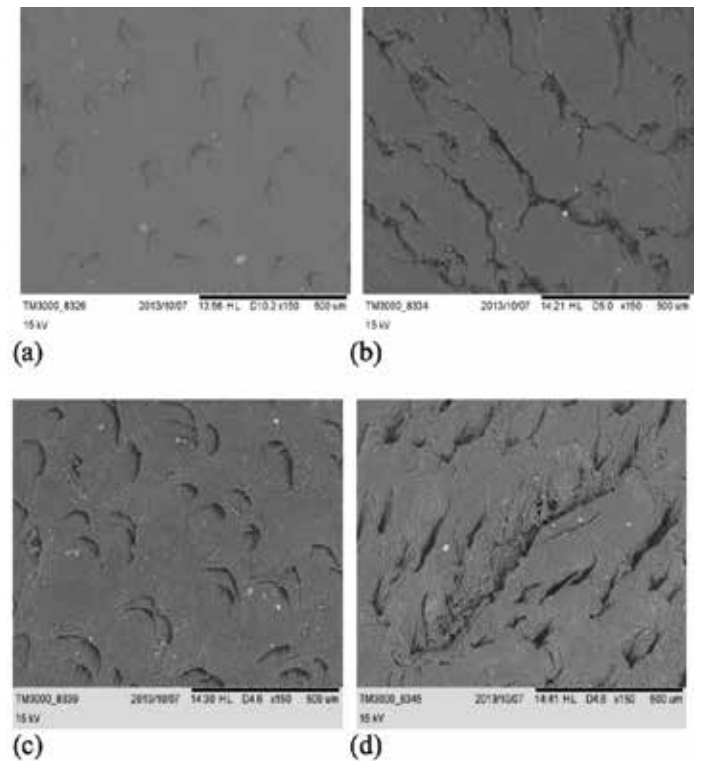


Figure 11. Vegetable-tanned leather samples (a) uncontaminated, (b) contaminated with *Aspergillus niger*, (c) *A. flavus*, and (d) *Penicillium herguei*.

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