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The Effect of Antibiotics (Streptomycin and Penicillin) in Ethanol Mist on the Surfaces of Model and Historical Leather from the Auschwitz-Birkenau State Museum

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Abstract

The aim of the study was to investigate the effect of disinfection with penicillin and/or streptomycin, added to ethanol mist, on the surface properties of both model and historical leather materials from the collections of the Auschwitz-Birkenau State Museum (A-BSM) in Oświęcim, Poland. The experimental conditions involved application of 90% ethanol mist alone or with penicillin, streptomycin or a mixture of both antibiotics using an airbrush. Changes in the morphology, structure and chemical properties of the sample surfaces compared to non-exposed samples were evaluated using Scanning Electron Microscopy (SEM), confocal microscopy (CM) and X-ray Photoelectron Spectroscopy (XPS). Microscopic studies demonstrated that exposure to penicillin or the antibiotic mixture caused subtle smoothing and flattening of tested leathers and a significant reduction in contamination of biological and mineral origin. Decreases in fluorescence intensity and fluorescent layer thickness were also observed, which, according to the XPS results, may be caused by the removal of a large amount of surface deposits or the reveal of deeper leather layers that were previously covered with inorganic particles. Therefore, it can be concluded that the developed method of applying antibiotics in ethanol mist does not have any significant negative effect on the surface of model and historical leather.

Keywords: cultural heritage; conservation; leather; scanning electron microscopy; confocal microscopy; X-ray photoelectron spectroscopy; disinfection; ethanol; antibiotics

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1. Introduction

The Auschwitz-Birkenau Memorial and Museum cares for the relics remaining from the German Nazi concentration and extermination camp. Auschwitz was a concentration camp complex, the two largest sections of which operated in the town of Oświęcim (Auschwitz) and the village of Brzezinka (Birkenau) in southern Poland. It operated from 1940 to 1945, and from 1942 onward, the complex also served as an extermination center where the "Final Solution to the Jewish Question" was carried out [1]. The camp served

several functions, and its history is complex. For this reason, it has universal significance and relates generally to the memory and history of the nations from which the victims came. The number of over a million victims, the extensive range of areas from which they were deported, and the industrial methods of extermination have made Auschwitz a symbol of the Holocaust [2].

Items brought to the camp by deportees, such as piles of clothes, shoes, suitcases, and everyday metal objects, and everything found in the camp after liberation, thanks to the efforts of former prisoners, were secured and preserved. These items form the core of the collections kept at the Auschwitz-Birkenau State Museum (A-BSM) currently. The A-BSM collections include approximately 95,000 items, 40 m^3 of civilian footwear, 40 m^3 of alloy metal objects from burned warehouses containing property stolen from deportees from the so-called Canada in Auschwitz II-Birkenau, and 2550 kg of objects, including human hair and single-use items such as glasses, umbrellas, screws, buttons, thread, etc. [3]. Many of these objects are made entirely or partially of leather. These include shoes, parts of suitcases, fragments of prostheses and orthoses, handbags, wallets and other small items (Figure 1).

Figure 1. Examples of leather objects from the A-BSM collection: (**A**) a pair of children's shoes, (**B**) a left foot prosthesis (photographers: (**A**) Nel Jastrzębiowska, (**B**) Jerzy Bestyński).

Historical objects, a testament to humanity's cultural and historical heritage, are subject to constant degradation. One significant factor influencing their state of preservation is biodeterioration, a complex process of biological destruction of materials caused by microorganisms. This phenomenon encompasses both mechanical and chemical interactions, leading to structural and esthetic changes in artifacts made of various materials: from stone, wood and metal to fabrics, paper and leather [4]. Historical leather objects, such as clothing, footwear, book bindings, belts, bags and military items, constitute a particularly sensitive group of artifacts. Leather, as an organic material, is highly susceptible to the activity of microorganisms, especially fungi and bacteria. Biodeterioration processes may lead to loss of elasticity, cracking, discoloration and even complete destruction of the collagen fiber structure. Conditions of elevated relative humidity and the presence of residual fats, tannins or preservatives, which may provide a source of nutrients for microflora, are particularly dangerous [5]. Understanding the mechanisms of biological deterioration and identifying the factors that determine it provide the basis for developing effective conservation and preventative methods that protect cultural heritage from irreversible damage.

To prevent biodeterioration, it is essential to implement appropriate preventive measures against microbiological attack, regularly monitor museum collections and use disinfection methods that are safe for the surfaces of historical objects. There are many methods for disinfecting historical objects, including both chemical techniques (e.g., quaternary ammonium salts, azoles, essential oils, nanometals, ethylene oxide) and physical tech-

niques (such as exposure to high or low temperatures, pressure, altered atmosphere, or radiation). Despite their effectiveness, each of these methods has certain drawbacks—they can affect the properties of the material, leading to changes in pH, color, and structure, as well as trigger processes such as depolymerization, hydrolysis, acidolysis, and accelerated aging. Furthermore, some of these methods may pose a health risk or be unsuitable for large-scale use [6–8].

For many years, the A-BSM laboratory has been conducting advanced research on safe and effective disinfection methods for use on historical objects. Recently, surface decontamination was tested using 90% ethanol mist (EM) sprayed under controlled conditions using an airbrush. This technique has proven to be exceptionally effective in eliminating non-spore-forming bacteria and fungi from both textiles and leather materials without causing structural changes [9,10]. Despite these promising results, tests showed that EM was not sufficiently effective against spore-forming bacteria of the genus *Bacillus*. In response to this limitation, the A-BSM laboratory team decided to expand the approach by combining ethanol mist with selected antibiotics to enhance its biocidal efficacy while maintaining safety for the disinfected objects. Penicillin and streptomycin are antibiotics with different mechanisms of action, which also exhibit a synergistic effect—penicillin disrupts the bacterial cell wall, allowing streptomycin to inhibit protein synthesis. For this reason, they are often used together to treat and prevent infections, for example, in cell cultures [11]. Taking advantage of the properties of penicillin and streptomycin, an attempt was made to decontaminate the surfaces of historical leather using ethanol mist enriched with these antibiotics (Figure 2).

Figure 2. Application of ethanol mist with antibiotics using an airbrush.

Due to the need to ensure the integrity of historical materials after the application of disinfectants, the tested method was checked for changes in the condition and possible damage to historical leather objects. The aim of this study was to assess the effect of ethanol mist with the addition of penicillin and streptomycin on the morphology, structure and chemical properties of surfaces of model and historical leather materials from the A-BSM collection.

2. Materials and Methods

2.1. Leather Materials

The test samples consisted of two types of leather. An element of a historical shoe from the early 20th century, originating from the A-BSM collection, was made available for testing by conservators and referred to as historical leather. Complementary studies were carried out using new, modern vegetable-tanned pig grain leather, hereinafter referred to as model leather. Pieces of model leather were cut into 40 × 40 mm samples, and historical leather into 30 × 30 mm samples (Figure 3).

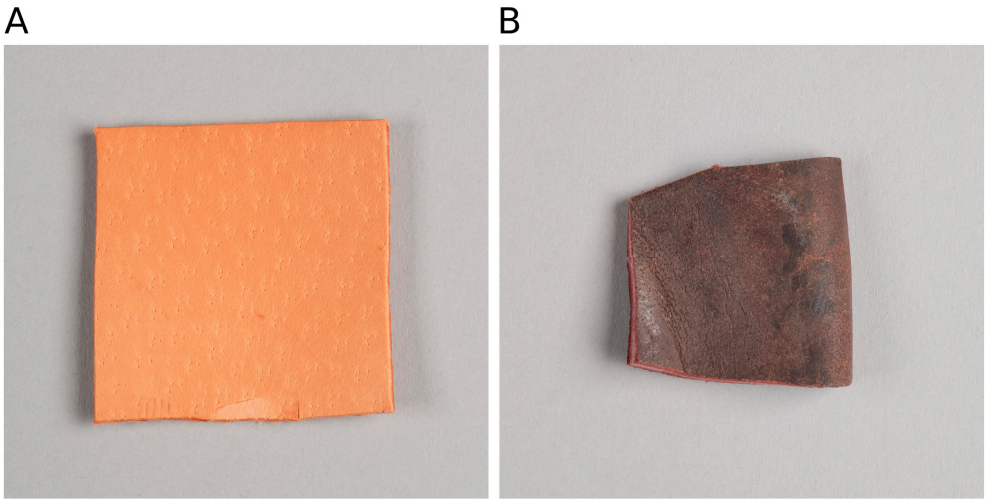

Figure 3. Leather samples used in the research: (**A**) model leather, (**B**) historical leather.

2.2. Ethanol and Antibiotics

Two types of antibiotics were used in the study: Penicillinum Procainicum L (TZF Polfa S.A., Warszawa, Poland), containing procaine lecithin benzylpenicillin (procaine penicillin G) as the active ingredient, and Crystalline Streptomycin Sulfate IM (Ibrahim Etem, Istanbul, Turkey). Both preparations were suspended in 90% ethyl alcohol (Chempur, Piekary Śląskie, Poland) and used in this form for testing.

2.3. Application of Ethanol in the Mist Form with Antibiotics

The technique of applying ethanol in the mist form (EM) to the surfaces of historical objects was developed, optimized, and documented previously [9,10]. Antibiotics suspended in ethanol were applied using a VE 0707 airbrush (Paasche Airbrush Co, Kenosha, WI, USA) operating at a pressure of 0.2 MPa and equipped with a PA HEAD VLH-5 nozzle with a 1.05 mm diameter tip. During application, a distance of 16 cm was maintained between the airbrush and the sample surface, and the application time was approximately 4 s per 100 cm^2. After application, the leather samples were placed in Petri dishes, tightly wrapped in polyethylene (PE) foil, and stored at room temperature for approximately 22 h. Storing the disinfected material for 22 h significantly improves the biocidal effectiveness of the ethanol mist itself, as demonstrated by the results of previous studies [9]. The tested samples were exposed to ethanol mist alone (EM) and ethanol mist enriched with penicillin (EM+P) or streptomycin (EM+S) individually and in combination (Mix) at concentrations shown in Table 1. The antibiotic doses used represent the highest concentrations of penicillin and streptomycin tested so far in the A-BSM laboratory, which are also the most biocidally effective (unpublished data).

Table 1. Concentrations and masses of ethanol and antibiotic solutions applied to the samples in the studies.

Sample Name	Concentration of Antibiotics [mg/L]		Mass of Applied Solution [g]	Mass of Antibiotics [μg/cm^2]	
	Penicillin	Streptomycin		Penicillin	Streptomycin
Model leather					
Control	0	0	0	0	0
EM	0	0	0.103	0	0
EM+P	4096	0	0.110	34.07	0
EM+S	0	512	0.093	0	3.60
EM+Mix	4096	512	0.181	56.06	7.01
Historical leather					
Control	0	0	0	0	0
EM	0	0	0.101	0	0
EM+P	4096	0	0.080	44.05	0
EM+S	0	512	0.049	0	3.37
EM+Mix	4096	512	0.059	32.48	4.06

2.4. Evaluation of the Effect of Ethanol Mist with Antibiotics on Leather Surface Properties

To evaluate the effect of the disinfection method used on the morphology, structure and chemical properties of the surfaces, a comparative analysis of model and historical leather samples was conducted using several research techniques.

2.4.1. SEM

The morphology of the leather samples was studied using scanning electron microscopy (SEM) by a high-resolution electron microscope JSM-7500F (Jeol Ltd, Akishima, Japan) with a cold field emission canal. The microscope was equipped with a retractable backscattered electron detector (RBEI) and Energy Dispersive X-Ray Spectroscopy (EDS) detector AZtecLiveLite Xplore 30 (Oxford Instruments, London, UK) with an Aztec Live Oxford Instruments NanoAnalysis software version 5.1 for EDS analysis. Before measurement, the samples were glued to holders and then placed individually on a dedicated measuring table. After the samples were introduced into the vacuum lock, they were evacuated. During the analysis, the vacuum pressure was maintained at approximately 9.6×10^{-5} mbar. Microscopic elemental composition measurements were performed. The samples were then removed from the microscope and coated with a 50-nanometer layer of gold using a vacuum sputter coater Emitech K575X (Quorum Technologies Ltd, Laughton, UK). After being placed back in the microscope, imaging was performed. Approximately 20 images were captured for each sample at various magnifications: 100×, 150×, 250×, 500×, 1000×, 2500×, 5000×, 10,000× and 25,000×, using an accelerating voltage of 15 kV.

2.4.2. CM

Samples of both model and historical leather were analyzed using the LSM 780 (Carl Zeiss AG, Oberkochen, Germany) fluorescence confocal microscope (CM), leveraging the autofluorescence properties of components such as collagen, proteins, fats, pigments and dyes present in the materials. Fluorescence emission spectra were captured using the 'Lambda mode' function, which utilizes a 34-channel detector to record emissions. The samples were illuminated with lasers at wavelengths of 355 nm and fluorescence emission was collected in 8.6 nm intervals across a spectral range of 400 nm to 700 nm using a 10×/0.45 Plan Achromat objective. Additionally, the confocal microscope was employed to obtain 3D images of the materials to analyze the thickness and structure of the surface of

the leader using the ortho view option, the function which allows to display a Z-Stack of images in an orthogonal view and measure distances in three dimensions.

2.4.3. XPS

X-ray photoelectron spectroscopy (XPS) analyses were performed using a multi-chamber ultra-high vacuum system equipped with a hemispherical energy analyzer SES R4000 (Gammadata Scienta AB, Uppsala, Sweden) and a non-monochromatic Mg Kα X-ray source (12 kV, 10 mA). The base pressure in the analytical chamber was 3×10^{-10} mbar, and during measurements approximately 2×10^{-8} mbar. The analyzed sample area was 5×0.8 mm^2. Detailed information regarding the acquisition, calibration and processing of XPS spectra is available in a previous publication [12]. Due to the low electrical conductivity of the tested materials, calibration was carried out using the aliphatic (C–C/C–H) component of the C 1s spectrum, whose binding energy (BE) was set to 285.0 eV.

XPS survey spectra analysis was used to determine the chemical composition of the surface of the tested samples. The total atomic concentration of elements was calculated using the CasaXPS software 2.3.23PR1.0 and the RSF coefficients provided therein for a layer several nanometers thick. Unfortunately, it was not possible to estimate this value more accurately without knowing the density of the material and its structure/texture. The calculations considered the signal from 95% of the photoelectrons emitted from a given surface.

3. Results

The aim of the study was to detect potential changes in physicochemical properties in model and historical leather samples following exposure to ethanol applied as a mist and the antibiotics penicillin and streptomycin.

3.1. Scanning Electron Microscopy Analysis of the Leather Samples

Morphological examination of the model leather revealed its porous nature with visible fibers. A granular layer with holes remaining after hair removal, arranged in triangular groups, is visible on the leather surface. The collagen fiber structure is visible through the hair holes. Comparison of the model leather images: the control sample with the samples exposed to ethanol and antibiotics (Figure 4) revealed a subtle thinning of the granular layer and a flattening of the leather structure, greatest after exposure to penicillin solution (Figure 4C) and the antibiotic mixture (Figure 4E).

Morphological examination of the historical leather samples revealed that this material was significantly degraded, likely due to the passage of time, dust, and coating with protective agents (Figure 5). Compared to the model leather, no hair holes or collagen fibers were visible on the surface of the historical leather. The control sample of historical leather was significantly contaminated with biological material in the form of fungal spores, plant pollen and aluminosilicates, as evidenced by the presence of clearly visible, crystallized grains with a plate-like and nodular shape. Analysis of the elemental composition using EDS confirmed the presence of the following elements: Si, Al, Mg, K, Na, Ca, and trace amounts of S, Fe, and Ti in the micro-areas of all tested historical samples. This indicates contamination of the leather with common aluminosilicates. Traces of Ca, Cl and Cr were also observed in samples exposed to ethanol mist alone and/or ethanol mist with antibiotics (Figure S1 in Supplementary Materials).

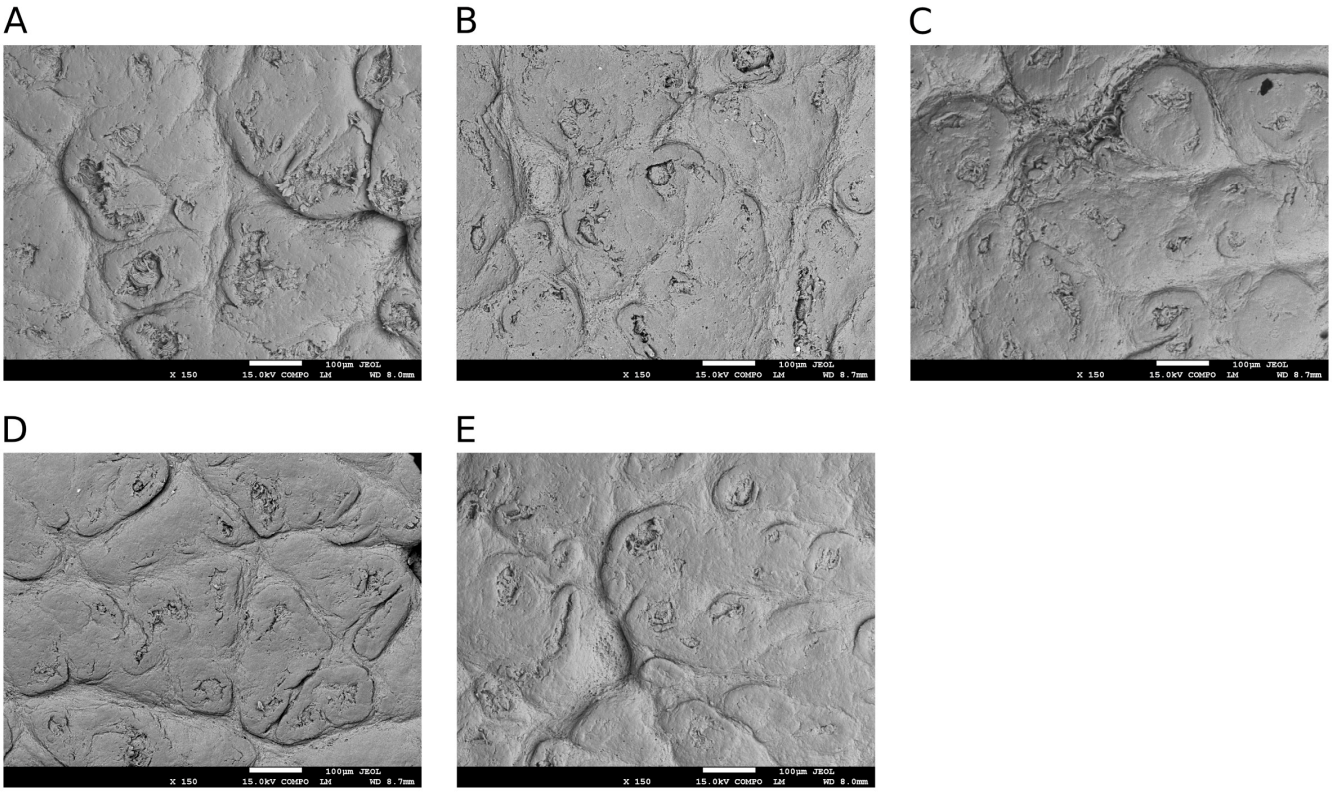

Figure 4. SEM images of the model leather samples: (**A**) control, subjected to: (**B**) EM, (**C**) EM+P, (**D**) EM+S, (**E**) EM+Mix (magnification 150×).

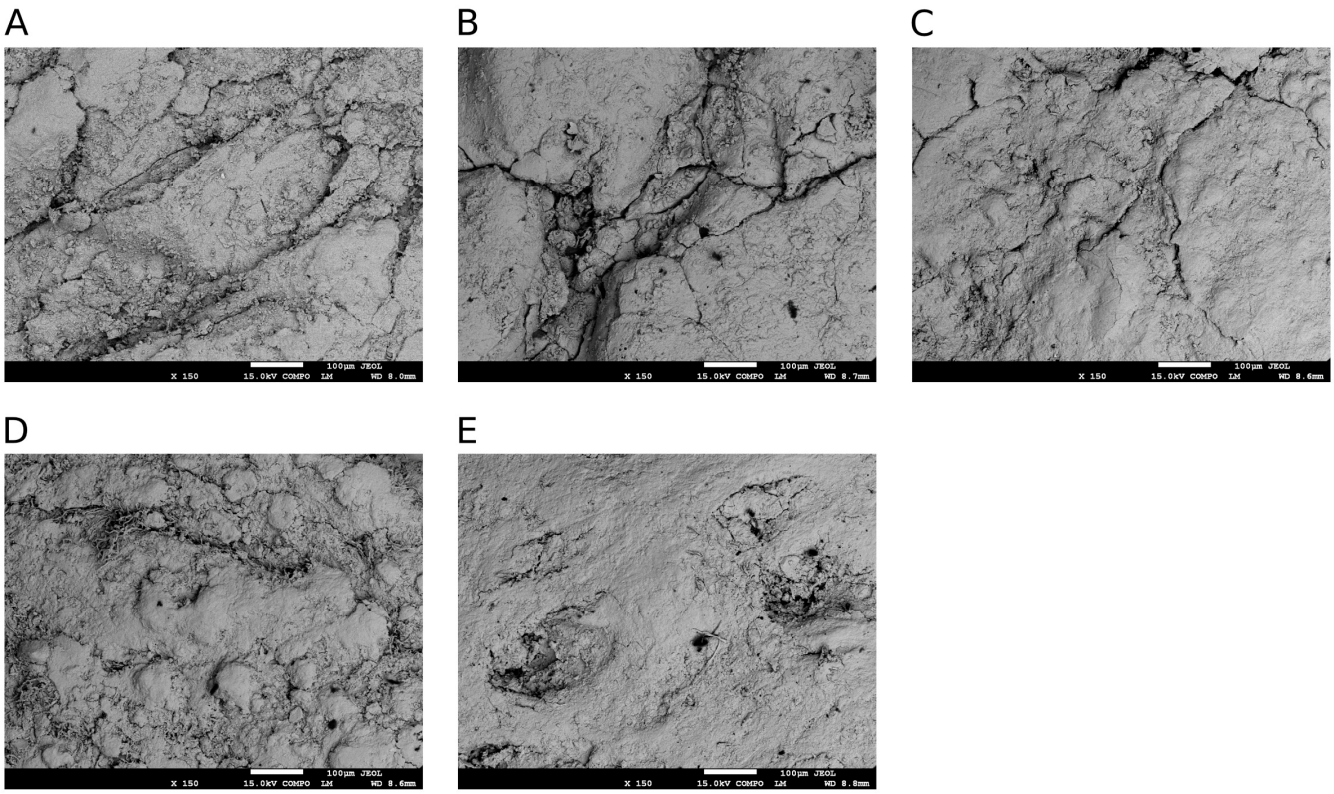

Figure 5. SEM images of the historical leather samples: (**A**) control, subjected to: (**B**) EM, (**C**) EM+P, (**D**) EM+S, (**E**) EM+Mix (magnification 150×).

Ethanol mist application resulted in the removal of some clay and reduced the amount of biological material and fungal spores (Figure 5B). This effect is primarily visible on flat surfaces. Both clay grains and biological material are still observed in the crevices of the sample material. The study indicates that the use of antibiotics in ethanol mist, i.e., streptomycin or penicillin solution, did not eliminate aluminosilicate contamination from the leather surface. Exposure to streptomycin or penicillin, however, had a positive effect in reducing the amount of biological material and fungal spores on the leather surface. In particular, after the application of ethanol with penicillin, both the leather surface and the crevices, recesses, and dents were covered with less biological material (sporangia/fungal spores) compared to the control sample (Figure 5C). An effect similar to that of penicillin was observed for historical leather samples subjected to a mixture of antibiotics (Figure 5E). These samples also contained a small amount of aluminosilicate contamination in the examined micro-areas, and the leather surface was less covered with biological material (fungal sporangia/spores) compared to the control sample and the remaining samples exposed to each antibiotic individually. Microscopic examinations showed that the use of penicillin or a mixture of antibiotics resulted in a more compact and smooth leather structure.

3.2. Analysis of Leather Samples Using Confocal Microscopy

Fluorescence emission spectra were obtained for model leather (Figure 6A) and historical leather (Figure 6B) using laser excitation at a wavelength of 355 nm and emission in the range of 400 to 700 nm.

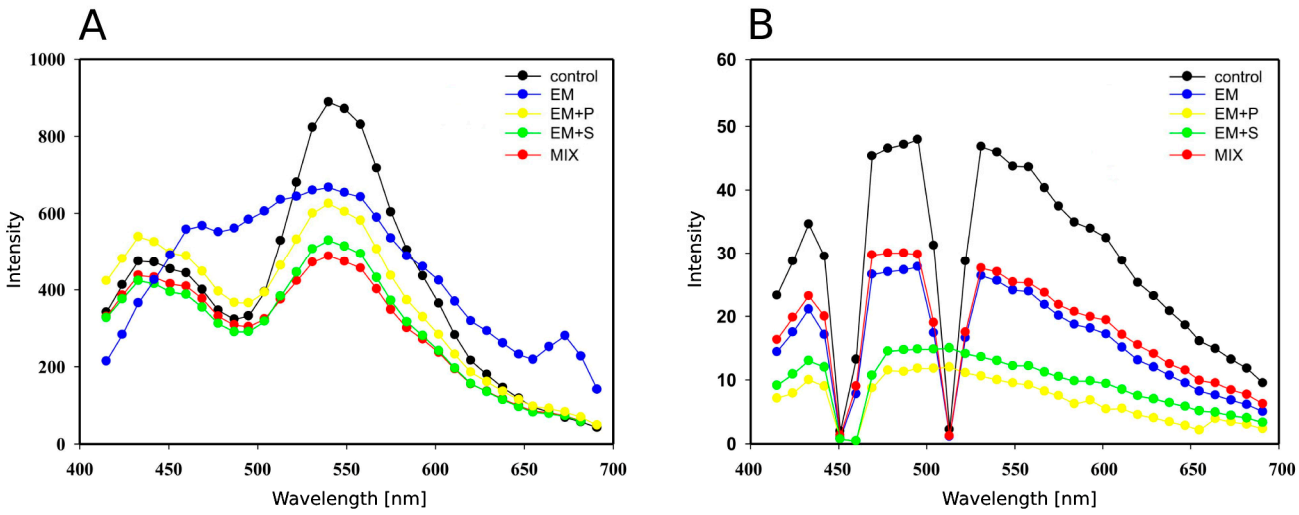

Figure 6. Fluorescence emission spectra of samples after excitation with light at a wavelength of 355 nm: (**A**) model leather, (**B**) historical leather.

As can be seen in Figure 6A, the fluorescence emission spectra for the control sample of model leather and for samples exposed to ethanol mist or antibiotics in ethanol mist show two distinct maxima: the first at approximately 430 nm and the second at 540 nm. Exposure of model leather samples to ethanol mist or ethanol mist containing antibiotics reduces the intensity of fluorescence. The greatest changes were observed in samples exposed to a mixture of antibiotics (Figure 6A, red line) and streptomycin in ethanol (Figure 6A, green line). The smallest decrease in fluorescence intensity was observed in the sample exposed to ethanol alone (Figure 6A, blue line). Additionally, in this case, the first maximum shifted towards red light by 355 nm.

The 3D images of model leather samples obtained using a confocal microscope at 100× magnification reveal a clear leather structure with hair follicles (Figure S2 in Supplementary

Materials). It can also be seen that the surface of the model leather sample changes slightly under the influence of ethanol mist or antibiotics suspended in it. The leather becomes smoother and less grainy, which is confirmed by measurements in the orthogonal view (Figure 7).

Analysis of orthogonal images revealed that the surface of the model leather exposed to ethanol mist and ethanol mist with antibiotics is slightly flattened. The cross-section perpendicular to the leather surface (z-axis) decreased from 190 μm for the control sample to 100 μm for the sample exposed to ethanol mist enriched with the mixture of antibiotics (Figure 7, windows on the right and above the main images). The thickness of the fluorescent layer itself decreased from 57 μm for the control sample of model leather to approximately 30 μm for samples exposed to ethanol mist and ethanol mist containing antibiotics (Figure 7, bright colorful stripes in the windows on the right and above the main images).

The shape of the spectrum for historical leather differs significantly from the typical spectrum of pure collagen. When excited by a 355 nm wavelength, three maxima can be distinguished at 450 nm, 495 nm and 530 nm, indicating the presence of additional components on the leather surface, even if these are only dyes or pigments used for coloring. The age of the leather also affects the shape of the spectrum, as collagen fibers undergo modification over time due to factors such as weather conditions, contact with the environment, and various types of pollution. In the case of historical leather materials, the control samples showed the highest fluorescence intensity as shown in Figure 6B. The application of ethanol mist, penicillin and streptomycin, as well as their mixture, caused a decrease in fluorescence intensity in the following order: EM+Mix > EM > EM+S > EM+P. The greatest decrease in fluorescence intensity was observed for EM+P.

Three-dimensional images of historical leather exposed to ethanol mist and a mixture of ethanol and antibiotics and leather that has not been exposed to any agent are shown in Figure S3 in Supplementary Materials. At first glance, the differences between the model leather and the historical leather are clear. The latter is much smoother than the model leather and does not have the characteristic hair follicles present on its surface. This is probably due to intensive use, age, and dirt. Analysis of these images showed slight changes in the surface structure of the leather after exposure to ethanol or antibiotics. As in the case of the previously studied model leather, the historical leather became smoother, as also shown by the analysis of orthogonal images (Figure 8).

As with the model leather, analysis of orthogonal view images of the historical leather revealed that its surface was slightly flatter after application of ethanol mist or ethanol mist containing antibiotics. The cross-section perpendicular to the leather surface (z-axis) decreased from 230 μm for the control sample to 190 μm for the EM+Mix sample, 100 μm for the EM+S sample, 80 μm for the EM sample and 38 μm for the EM+P sample (Figure 8, windows on the right and above the main images). The thickness of the fluorescent layer itself decreased from 24 μm for the historical leather control sample to 17 μm for the EM sample and the EM+Mix sample, 13 μm for the EM+P sample and 20 μm for the EM+S sample (Figure 8, bright colorful stripes in the windows on the right and above the main images).

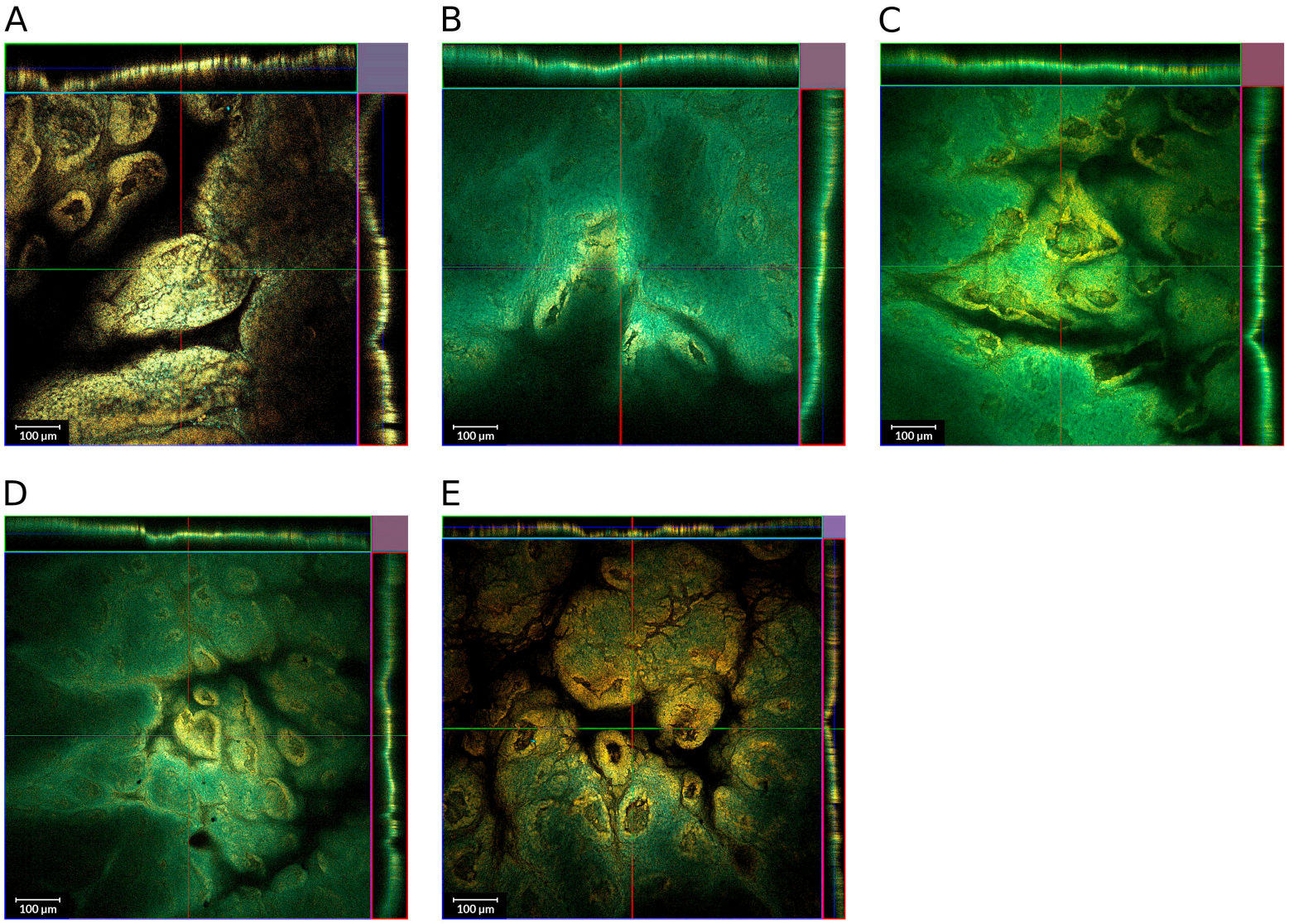

Figure 7. Confocal images of the model leather samples in orthogonal view mode: (**A**) control, subjected to: (**B**) EM, (**C**) EM+P, (**D**) EM+S, (**E**) EM+Mix (magnification 100×).

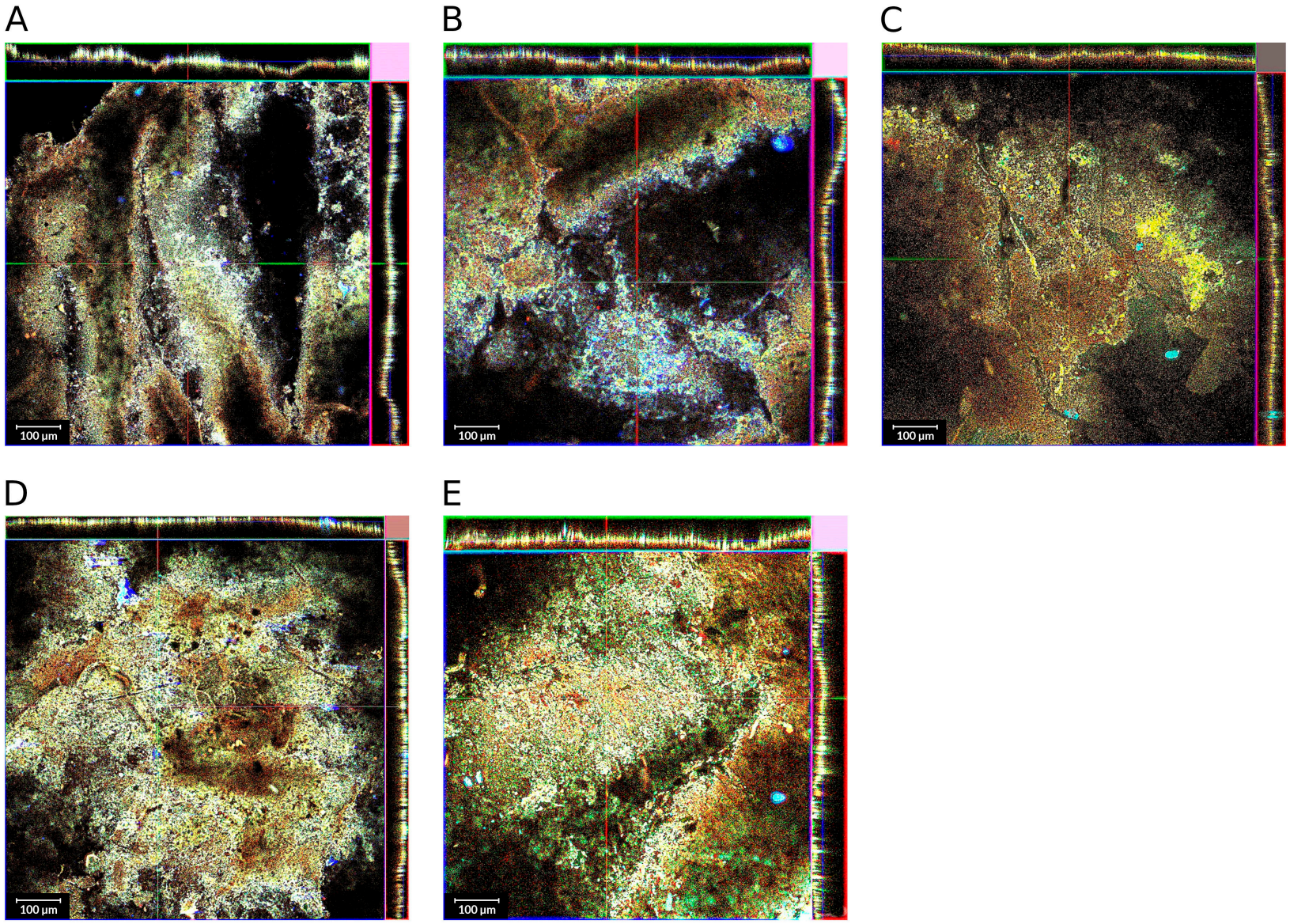

Figure 8. Confocal images of the historical leather samples in orthogonal view mode: (**A**) control, subjected to: (**B**) EM, (**C**) EM+P, (**D**) EM+S, (**E**) EM+Mix (magnification 100×).

3.3. Analysis of Leather Samples Using the XPS Technique

When examining historical artifacts, it is important to remember that many of them are covered with protective layers that are not part of the original material. This complicates the assessment of the impact of disinfectants on these artifacts. The key question is whether these substances affect only the surface coatings or penetrate deeper, potentially affecting the original substrate. X-ray Photoelectron Spectroscopy (XPS) is particularly well suited to addressing this issue. By analyzing a few nanometers of the surface, XPS can detect subtle chemical changes that indicate whether the historical material underneath has been altered. This makes it a crucial technique in the field of conservation, where protecting the authenticity and condition of cultural heritage objects is a top priority.

In the case of model leather, carbon and oxygen were present in all tested samples, which is natural for samples prepared in an air atmosphere (Table 2). In addition, each sample contained traces of calcium in the form of sulfates and/or carbonates (BE of the Ca $2p_{3/2}$ line ~348 eV), probably originating from water. All model leather samples contain sulfur and nitrogen. The binding energy of the S $2p_{3/2}$ line, ~168 eV, suggests sulfates. Some samples contain small amounts of sodium and silicon. No aluminum is observed. Considering the chemical composition of the surface, application of ethanol mist with antibiotics causes very slight changes to the model leathers. While maintaining the same amounts of carbon, oxygen, calcium, and nitrogen, changes can only be observed in the amounts of silicon, sodium, and sulfur (Table 2). This is most likely related to the mechanical removal of natural dirt. However, it is worth mentioning that the above minor differences in the chemical composition of the surface may also result from the fact that leather samples taken from different parts of the material may have differed slightly in the amount of inorganic dirt already present before exposure to the process.

Table 2. Chemical composition of model leather surfaces determined by XPS.

Name of Sample	Elemental Composition [% at.]							
	C	O	Ca	N	S	Na	Si	Al
control	79.75	16.09	0.49	2.59	0.94	0.13	---	---
EM	79.15	16.42	0.52	2.40	0.70	0.18	0.64	---
EM+P	78.16	17.63	0.40	2.97	0.69	0.17	---	---
EM+S	83.87	12.49	0.43	2.12	0.61	---	0.48	---
EM+Mix	83.92	12.23	0.42	2.25	0.64	---	0.54	---

The historical leather samples examined have carbon and oxygen on their surface, which is natural for samples stored for many years in the air. In addition, they contain traces of calcium (in the form of sulfates and/or carbonates), probably originating from water. Apart from the above-mentioned elements, nitrogen, sulfur, aluminum, and silicon are also present. Silicon and aluminum come from sand (silica) and clay (aluminosilicates) contamination. Nitrogen and sulfur, in addition to their natural sources (skin, hair, hormones, amino acids), may come from the use of penicillin (S and N) and streptomycin (N). Trace amounts of NaCl were detected in the EM+Mix sample (Table 3). The amount of silicon and aluminum observed for the EM+Mix and EM+P samples was more than twice as high as for the other samples. The lowest amounts of nitrogen, oxygen, and sulfur were recorded for the EM+S sample. No increase in nitrogen and sulfur content was observed in the tested model and historical leather samples, which could indicate surface modifications related to the application of penicillin and/or streptomycin.

Table 3. Chemical composition of historical leather surfaces determined by XPS.

Name of Sample	Elemental Composition [% at.]						
	C	O	Ca	N	S	Si	Al
control	77.73	15.55	0.99	2.32	0.65	1.61	1.15
EM	81.11	14.21	0.65	1.30	0.62	1.24	0.87
EM+P	72.07	17.81	1.05	1.93	0.56	4.18	2.39
EM+S	88.11	7.89	0.52	0.32	0.38	1.89	0.88
EM+Mix	65.11	22.89	0.89	1.75	1.14	4.43	2.92

Despite the fact that tested processes do not affect the quantitative changes in elements on the surface of the tested leathers, in order to exclude any qualitative changes, additional high-resolution XPS spectra were recorded in the C 1s, O 1s, and N 1s binding energy regions (Tables S1–S6).

The C 1s spectra of the tested samples (Figure 9) reveal a number of functional groups containing carbon bonds: alkene (C=C) and alkyl (C–C/C–H), as well as bonds indicating the presence of oxidized carbon groups: alkoxy (C–O) and carboxyl (O–C=O). The model leather samples also have carbonate groups, and significantly more alkoxy groups can be observed. In addition to the above-mentioned functional groups, it should be noted that –NH–C=O bonds give a signal with an energy of approximately 288 eV, while C–N bonds occur at energies characteristic of C–O bonds. The amounts of carbon functional groups within the tested group of samples vary very little (Tables S1 and S2), which indicates that exposure to ethanol mist with antibiotics has no effect on the surface condition.

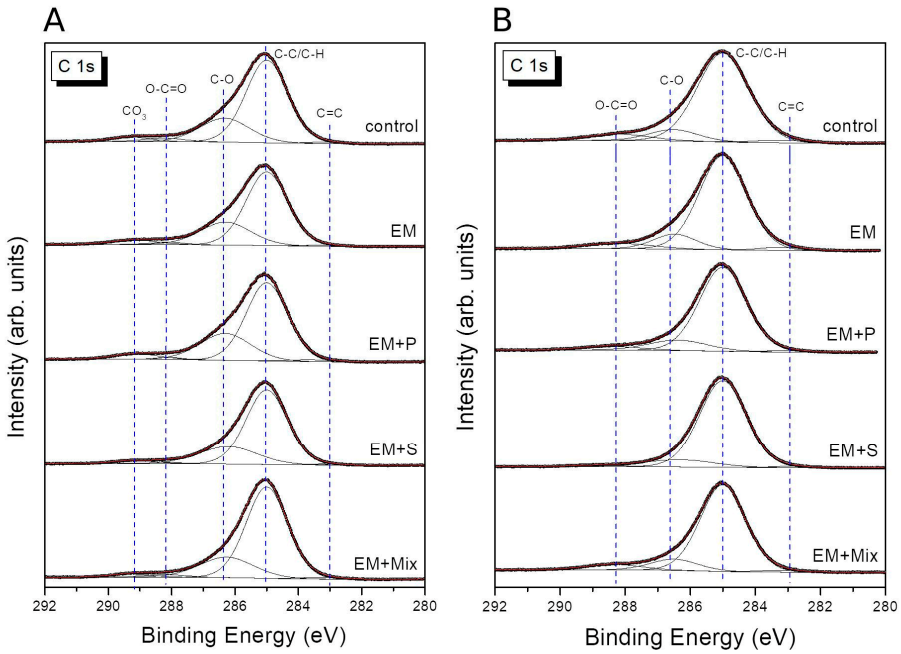

Figure 9. XPS spectra of the C 1s line for control samples and samples subjected to EM, EM+P, EM+S and EM+Mix: (**A**) model samples and (**B**) historical samples.

The dominant group in all O 1s spectra (Figure 10) is the hydroxyl group (>92%). The remaining few percent are carboxyl groups in the case of model leathers and ether groups in the case of historical leathers. Subjecting the samples to ethanol mist alone or with antibiotics does not cause significant changes in the quantitative image of oxygen functional groups (Tables S3 and S4).

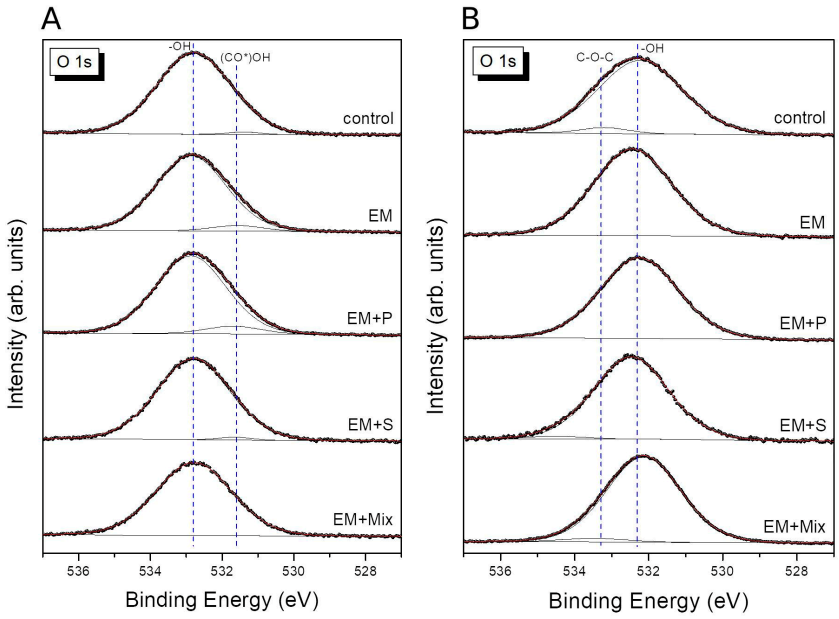

Figure 10. XPS spectra of the O 1s line for control samples and samples subjected to EM, EM+P, EM+S and EM+Mix: (**A**) model samples and (**B**) historical samples.

Due to the low amount of nitrogen present on the surface of the samples, the analysis of the obtained high-resolution N 1s spectra is subject to a significantly higher error and therefore is not presented here. More details can be found in the Supplementary Materials (Tables S5 and S6).

In summary, exposure to ethanol mist with antibiotics does not cause significant changes in carbon, oxygen and nitrogen functional groups. Minor changes are observed in oxygen functional groups, which may indicate the removal of inorganic contaminants.

4. Discussion

Ensuring microbiological cleanliness of objects is a necessary condition for initiating conservation works at the Auschwitz-Birkenau State Museum (A-BSM). Studies conducted by the A-BSM laboratory team confirmed the presence of microorganisms on historical leather objects from the A-BSM collection. They may accelerate degradation of these materials and also pose a health risk to conservators [10]. Therefore, it is crucial to develop a disinfection method that effectively eliminates microorganisms while avoiding damage to objects.

Scientific reports indicate that various antibiotics have been investigated as a means of eliminating microorganisms responsible for the biodeterioration of cultural heritage objects, but their effectiveness was variable. In one study, 13 antibiotics failed to control resistant *Streptomyces* bacteria on wall paintings in Egyptian tombs at Tell Basta and Tanis, necessitating the use of gamma radiation [13]. In another case, the antibiotic 6-pentyl-α-pyronephenol, produced by *Trichoderma* fungi, effectively eliminated *Aspergillus niger* and *Aspergillus flavus* fungi on wall paintings in the Nfer Bau Ptah Tomb in Giza [14]. Mitomycin C successfully removed only one of three tested bacterial species (*Blastococcus saxobsidens*) responsible for the biodeterioration of stone objects [15]. Meanwhile, in Pompeii, amoxicillin was able to eliminate *Streptococcus* bacteria from ancient frescoes in the Villa of the Mysteries [16].

In fields beyond cultural heritage, researchers also focus on leather antisepsis. There is a need to identify effective antibacterial agents in the footwear industry to increase the quality of shoes that do not pose a hazard to humans and to the natural environment. The use of oregano essential oil was tested at the stage of fatliquoring or by spraying of leather, and its

antifungal and antibacterial properties were confirmed [17]. Also, covering leather with Ag or Ag–TiO_2 nanoparticles was biocidally effective and did not cause harm to the structure of leather [18,19]. Moreover, combinations of certain commonly used clinical antibiotics demonstrate excellent effectiveness for short-term hide preservation [20]. Abdulhusein et al. provided a comprehensive review of antibacterial agents used in the leather industry but mentioned only one antibiotic, i.e., ciproflaxin, which was incorporated into polyurethane chains and used as a retanning agent in the leather manufacturing process [21,22].

In cultural heritage preservation, the most important aspect is the use of biocidal agents that are neutral toward the surfaces of objects. In previous years, the A-BSM laboratory team tested various innovative methods based on results from the medical field [23–25]. One of the first approaches involved the use of vaporized hydrogen peroxide (VHP), which demonstrated high efficacy in disinfecting historical materials, including leather. Unfortunately, the limited availability of specialized VHP generators hinders the broader implementation of this method [26]. Subsequent experiments showed the positive potential of using a diode laser, which effectively eliminated microorganisms from the surfaces of various types of leather without causing damage. However, it should be noted that this technology is mainly effective during the early stages of biodeterioration [27]. In the most recent studies, the A-BSM laboratory team demonstrated that exposure of another type of material, i.e., textiles, to penicillin and streptomycin in ethanol mist does not negatively affect the surface properties of historical cotton (unpublished). Therefore, an attempt was made to decontaminate the surfaces of historical leather using ethanol mist enriched with penicillin and streptomycin. The proposed new fogging methodology for leather disinfection is an innovative approach that combines the effectiveness of biocidal agents with a high level of safety and ease of use. This technique involves applying the product in a fine mist, enabling even coverage of the object's surface and reaching hard-to-reach areas without the need for direct contact with the surface. By utilizing advanced spraying technology, the fogging process significantly reduces disinfectant consumption, shortens application time, and minimizes the risk of irritation.

The aim of the present study was to determine potential changes in the morphology, structure, and chemical properties of model and historical leather after exposure to penicillin and/or streptomycin in ethanol mist. For this purpose, analytical techniques such as scanning electron microscopy (SEM), confocal microscopy (CM), and X-ray photoelectron spectroscopy (XPS) were applied. These investigations were based on the experience of the A-BSM laboratory team, which had previously employed these methods in numerous projects analyzing the effects of VHP, diode lasers and ethanol mist on heritage materials [10,26,27]. Two types of leather were tested to analyze both possible variations in the composition of the surface layer. The historic leather is coated with protective substances applied during conservation treatments, while the model leather is not. The use of the model leather simulates the situation if the object was not protected by chemical substances and the relevant historic matter (collagen) was directly exposed to external factors such as disinfectant.

Numerous research revealed that the SEM technique enables the assessment of potential disruptions in the surface fiber network and the emergence of gelatinized regions within the collagen fibers, and it is widely used in studies of the biodeterioration of historical objects [28–31]. The analysis conducted in these tests using SEM allowed for a detailed assessment of morphological changes resulting from the action of antibiotics. The most significant morphological changes were observed after exposure to penicillin and the antibiotic mixture in both model and historical leather. Exposure to ethanol mist in these variants caused subtle flattening of the model leather surface and smoothing of its grain layer. Microscopic studies demonstrated that exposure to penicillin or the antibiotic

mixture resulted in a more compact and smooth structure of historical leather. However, these were not significant visible changes and probably resulted from the mechanical cleaning effect of the airstream generated during airbrush application. Compared to the control sample (unexposed), whose surface was heavily covered with fungal spores and sporangia, plant pollen, and aluminosilicates, the samples subjected to penicillin and antibiotic mixture showed significantly lower contamination. The application of ethanol mist enriched with antibiotics markedly reduced the number of biological components on the surface of historical leather, particularly fungal spores and sporangia, which is beneficial for disinfection processes. This effect was especially visible after exposure to penicillin, whose action affected both the surface of leather and hard-to-reach microcrevices.

3D and orthogonal images obtained using confocal fluorescence microscopy, together with measurements of cross-sections and fluorescent layer thickness, confirmed that both model and historical leather samples exposed to the ethanol mist containing antibiotics are slightly flattened, similarly to the SEM observations. The greatest differences were observed in model leather subjected to the antibiotic mixture and historical leather exposed to penicillin. However, morphological changes on the surface of model leather were not very pronounced, likely due to its homogeneous and less complex composition. Despite similar minor changes in surface structure, slightly bigger differences in fluorescence intensity and the thickness of the fluorescent layer of historical leather were observed. This is probably because historical leather is old and heterogeneous, as confirmed by analysis of the fluorescence emission spectrum. The spectrum of historical leather differs significantly from that of typical pure collagen, indicating the presence of additional surface components, such as dyes used for coloring, or biological and mineral contaminants, as also visible in SEM images. The age of the leather may also influence the spectrum shape similarly as in the case of other organic substances [32], as collagen fibers undergo modifications over time due to atmospheric exposure, environmental contact, and accumulation of various types of dirt. Compared to control samples, changes in fluorescence intensity following exposure to ethanol and antibiotics are noticeable. Fluorescence in tested leathers may originate from various sources. Potential fluorophores include natural organic compounds (proteins, amino acids), products of protein and lipid degradation [33,34], residues of tanning agents and other substances used in leather processing (e.g., plant tannins), remnants of conservation treatments, and biological or environmental contaminants or their metabolites. A decrease in fluorescence intensity may indicate removal or deeper penetration of fluorophores into the material, degradation of organic material (e.g., tanning agents), light absorption by degradation products, dilution of conservation layers, or reduction in microbiological contamination [35]. Therefore, it is possible that the observed decrease in fluorescence intensity and fluorescent layer thickness results from the removal of surface contaminants from the historical leather due to the mechanical sweeping effect of the airstream generated during the application of ethanol mist with antibiotics using an airbrush, and is not a result of the action of the antibiotics themselves.

XPS analysis revealed that changes in the chemical composition of the model leather surface after disinfection were minimal. The absence of significant quantitative changes in the main elements and functional groups indicates that the disinfection processes do not chemically alter the surface structure of the leather. Variations in the content of sodium, silicon, aluminum, or sulfur can be attributed to the removal of loosely bound contaminants or differences in the initial content of inorganic impurities in the analyzed fragments. For historical leather, an interesting finding was the noticeably higher content of aluminum and silicon in samples exposed to the mixture of penicillin and streptomycin, as well as with penicillin alone. This may result from greater removal of surface deposits or exposure of deeper leather layers previously containing inorganic particles (e.g., environmental

dust). Alternatively, the presence of these elements may indicate contamination associated with the antibiotics themselves. The sample exposed to the ethanol mist enriched with streptomycin showed the lowest content of nitrogen, oxygen, and sulfur, which may reflect the lower ability of streptomycin to interact with the leather surface compared to penicillin. The content of carbon-, oxygen-, and nitrogen-containing functional groups on the surface remained practically unchanged after application of ethanol mist with antibiotics. This indicates the absence of chemical reactions leading to degradation or modification of the main protein structures of leather (e.g., collagen). Exposure of model and historical leather to penicillin, streptomycin, or their mixture suspended in ethanol mist does not cause significant changes in the chemical composition of the surface material (up to several nanometers). Changes in the content of inorganic elements are secondary and most likely result from the removal of loosely bound contaminants rather than chemical interactions between the antibiotics and the leather structure.

To examine changes on the material surface in as much detail as possible, several techniques that differ in measurement depth and type were used. Fluorescence measurements using confocal microscopy allow for the assessment of changes at a greater depth (on the order of μm) than XPS results (on the order of nm). XPS analysis results did not reveal significant changes in the chemical composition of the leather surface after applying ethanol mist with antibiotics, indicating that disinfection had no direct effect on the superficial layer. However, the results of studies conducted using confocal microscopy indicate a flattening of the surface and a reduction in the thickness of the fluorescent layer. The lack of changes in chemical composition, coupled with the change in the appearance of the samples, indicates the dominant effect of the airstream applied with the biocide. Most likely, the airstream from the airbrush removed and/or moved loosely bound contaminants covering the entire sample surface into the crevices.

It is worth emphasizing that the application of ethanol mist alone, without antibiotics, also resulted in slight surface smoothing, reduction in the fluorescent layer thickness, minimal changes in the content of inorganic elements in both tested leathers, and a decrease in biological and mineral surface contamination of historical leather. Furthermore, in the case of historical leather, ethanol mist caused a decrease in cross-section thickness and a reduction in fluorescence intensity of a magnitude between that observed with the antibiotic mixture and with penicillin. Therefore, it can be concluded that the developed method of applying penicillin and streptomycin in ethanol mist does not negatively affect the surface properties of historical leather objects. However, to recommend this method for disinfection of such objects at A-BSM, further studies are necessary to evaluate its biocidal efficacy against the typical microbiota present on historical leather objects from the A-BSM collection. In addition, the long-term effects of ethanol mist with antibiotics on material surfaces should be checked.

5. Conclusions

The observed subtle changes in the leather's surface properties are primarily due to the application technique used, i.e., treatment with an airstream containing ethanol mist. This application method removes surface contaminants by blowing them away. No changes directly resulting from the action of antibiotics or their residues were observed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app152212259/s1, Figure S1. Exemplary EDS spectra of the historical leather samples. Figure S2. Confocal images of the model leather samples: (A) control, subjected to: (B) EM, (C) EM+P, (D) EM+S, (E) EM+Mix (magnification 100×). Figure S3. Confocal images of the historical leather samples: (A) control, subjected to: (B) EM, (C) EM+P, (D) EM+S, (E) EM+Mix (magnification 100×). Table S1. XPS C 1s line of model leather. Table S2. XPS C 1s line of

historical leather. Table S3. XPS O 1s line of model leather. Table S4. XPS O 1s line of historical leather. Table S5. XPS N 1s line of model leather. Table S6. XPS N 1s line of historical leather.

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Abbreviations

The following abbreviations are used in this manuscript:

A-BSM	Auschwitz-Birkenau State Museum
CM	Confocal Microscopy
EDS	Energy Dispersive X-Ray Spectroscopy
EM	Ethanol Mist
EM+P	Ethanol Mist enriched with Penicillin
EM+S	Ethanol Mist enriched with Streptomycin
EM+Mix	Ethanol Mist enriched with a mixture of Penicillin and Streptomycin
SEM	Scanning Electron Microscopy
VHP	Vaporized Hydrogen Peroxide
XPS	X-ray Photoelectron Spectroscopy

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