Biocidal Activity of Selected Preparations for Leather Protection

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Abstract
This research work involved the selection of biocides for the antimicrobial treatment of leathers. The scope of the research work covered microbiological tests of leather samples tanned utilising the same technology, with microbiological treatment made using 9 different preparations, as well as a test of leather without biostabilisation. Commercial biocides taken for investigation were as follows: NS 500 – an aqueous solution of nanosilver 500 ppm, ODH – tea tree oil, and OP – orange oil. Preparations prepared by diluting the purchased products were as follows: S27-22 – an aqueous solution containing silver chloride and titanium dioxide, SH 22-27 – an aqueous solution containing zinc pyrithione, ST 99-19 – an aqueous solution containing dimethyltetradecyl [3-(trimethoxysilyl)propyl]ammonium chloride, SU – an aqueous solution containing polyhexamethylene biguanide, and SGP – a saturated solution of aluminum potassium sulfate. Also a preparation was made by combining NS 500 M250 – 500 ppm nanosilver solution and a 250 ppm copper colloid. Evaluation of the effectiveness of biostabilisation of the leather samples and antimicrobial activity of the preparations used was carried out. Staphylococcus aureus and Escherichia coli bacteria and Candida albicans, Aspergillus brasiliensis and Trichophyton mentagrophytes fungi were used in the study. The assessment of microbial growth on samples was carried out according to Polish standards. Assessment of the resistance to bacteria and C. albicans yeast was made according to the PN-EN ISO 20645: 2006 standard – a method of diffusion on an agar plate. Assessment of the resistance to A. brasiliensis and T. mentagrophytes fungi was made according to the PN-EN ISO 14119: 2005 standard. Among the tested preparations to protect leathers against the effects of microorganisms, overall efficacy was obtained for SH 22-27; NS 500 M 250, SU and ODH.

Key words: biocides, antimicrobial activity, biostabilisation, leather, antimicrobial leather protection.

Introduction
The protection of leather against microbes at different stages of the tanning process does not make it completely resistant to microflora. Leather, the result of the technological process of tanning, is a substrate for the growth of many types of microorganisms. Filamentous fungi cause major damage to finished leathers during storage and use. Fungi that are present in the form of spores and hyphae of mycelium cause the biodegradation of finished leather. Relative humidity during storage has a significant effect and stimulates mould growth on leathers. The availability of various sources of nutrients stimulates spores and hyphae to germinate, and then supports their growth. Getting a proper method of protection for leather against mould during production is very important; it depends on the tanning agents, oils, oil emulsions, fats and other auxiliary substances [1, 2].

Stains on finished leathers can be caused by decolourisation by metabolites of microorganisms. Azo dyes make up the majority of all dyes used in leather production. In general, the decolourisation of azo dyes can occur under the influence of conventional anaerobic bacteria, facultative anaerobic bacteria and various groups of bacteria under aerobic conditions. The mechanism of degradation of azo dyes by microorganisms involves the reductive breaking of azo bonds (-N = N-) with the azoreductase enzyme under anaerobic conditions, which leads to the formation of colourless solutions containing potentially dangerous aromatic amines [3, 4].

Finished leather intended for the production of footwear, leather fancy goods and carpets should be characterised by increased microbiological resistance, consisting in the reduction of colonising microorganisms during storage and use. This requires additional microbiological treatment with active substances. It is necessary that finished leather, and footwear made thereof be characterised by increased microbiological resistance, consisting in the protection of adequate microbiological comfort for the feet through significant inhibition of the growth of or reduction in microorganisms in its interior. Also, leather intended for the production of leather fancy goods and carpets should be protected with biocides to prevent the deposition of microorganisms on their surface, which will protect them against the harmful activity of microorganisms [5, 6].

Literature reports [7-12] indicate that the most common clinical form of mycosis is the infection of toenails and feet. The results of clinical trials of 21,666 patients with suspected skin mycosis and its appendages, carried out at the Dermatological Clinic in Gdańsk (Poland) in the years 2000-2011, revealed 5012 cases of positive culture results. Dermatophytes were cultured in 2445 cases, which accounted for 48.8% of all infections. Yeast-like fungus constituted 44.8%. The remaining 6.4% were infections caused by mould fungi and Malassezia spp. Among the dermatophytes, T. rubrum was the most often isolated – 46.8% and second T. mentagrophytes – 33.2%. The main clinical forms were: onychomycosis of the foot nail plates (45.77%) and nails of the hands (24.24%). In recent years a steady increase in fungal infections caused by yeast-like fungi has been observed. Infections of the foot nail plates were the most common clinical form of mycosis [13].

In tanneries, to obtain finished leathers with a hygienic finish, treatment with microbiologically active substances should be carried out, most preferably at the stage of finishing the leathers into ready-made ones. The choice of method of
applying biocides to leather depends on the economics of the microbial treatment process at the leather finishing stage as well as on the manufacturer’s potential and technical conditions. The use of biocides for the protection of materials should be controlled within the scope of the dose applied and intended use of the final product. Overuse of any bactericide or fungicide should not take place so as not to exert a negative influence on the environment, and also to prevent microorganism resistance to a given biocide [14-19].

Since microorganisms are found everywhere and can be transferred via air, water, food, etc., control and preventive measures are necessary to suppress their negative activity. Unfortunately, the widespread and irresponsible use of antibiotics and disinfectants has led to the development of resistant strains, causing difficulties in the treatment of infections caused by them [20].

Biocides used in the tanning industry are generally non-oxidising. They can be classified as quaternary ammonium compounds, isothiazoles, halogenated organic compounds, such as Bronopol (2-bromo-2-nitro-propane-1,3-diol), isothiazoles and halogenated organic compounds containing heterocycles, such as derivatives of benzothiazole [21]. Fungi- cides can be added to leather during lubrication to inhibit mould growth during storage and transportation [22].

Some synthetic fungicides are known to work, but when used to excess, they are a threat to the environment. In addition, microorganisms become resistant to some fungicides with time. To find alternative substances, Akpomie evaluated the antifungal activity of orange oil [23]. The results suggest that orange seed oil has great potential as a natural antimicrobial substance. Growth of all the microorganisms tested (A. niger, A. fumigatus, A. flavus, Paecilomyces sp., Penicillium spp., Mucor mucido, Rhizopus nigricans), with the exception of Alternaria sp., was inhibited by the orange oil.

In the study [23], two small pieces of leather (sterilised and contaminated) were placed in a 5% oil extract in an orbital shaker for 4 hours. In another study, samples of used and contaminated shoes were oiled with orange extract oil. Then, each sample was left in the open air and observed daily for the growth of fungi. Studies have shown that the Akpomie seed oil of the sweet orange has advantageous properties in the prevention of the deterioration of skin and leather products. The main objective of the research conducted by Širvaityte and others was to determine the applicability of the essential oil of thyme as an antimicrobial agent in the processing of leather into chrome tanned leather. Differences in the chemical composition of commercial and pure essential oils of thyme were determined. It was found that these differences affect the antimicrobial activity of essential oils. Gram-positive bacteria have proven to be more sensitive to the oils of thyme than Gram-negative bacteria. Pseudomonas aeruginosa showed low sensitivity to the essential oils of thyme selected, but the leather samples treated with these oils remained resistant to these bacteria. The antibacterial activity of essential oils of thyme depends on their chemical composition, mainly on the phenolic components. The most important phenol compounds are thymol and carvacrol. Oil of Thymus vulgaris (0.05 to 5 wt%) was added to the base oil used for oiling. The amount of oil used in the process must be higher than 3%. Smaller amounts (0.05% and 1.0%) showed no zone of inhibition, although skin samples remained resistant to the bacteria selected. The conventional synthetic preservative 2-thiocyanomethylthiobenzotiazole (TCMTB) used in 0.2% per weight of “wet-blue” did not protect leather better than 3-5 wt% of essential oil.

The essential oils of thyme can be used as a preservative in a mixture with synthetic biocide. In this case, the amount of synthetic biocide required may be reduced from 0.2% to 0.05% of the weight of wet-blue leather, and thyme oil to 3.75% of the weight of wet-blue leather [24, 25].

In China, a new nano-composite antimicrobial has been used for children’s shoe inserts, showing long-term effectiveness. The product contained 0.8 g/l of nano ZnO, 10 g/l of isothiazolinecetone, 0.05 g/l of Tween, and 3 g/l of acrylic resin; and the initial pH was 6.0. An antimicrobial agent was applied by spraying, whose method of addition is very important for its absorption and distribution. Assessment of the degree of inhibition was made with a quantitative method. After treatment with a 2% nano-composite, the antimicrobial insert may effectively inhibit 97% of the growth of the test fungi [26-28].

The Conceria Stefania company created and patented a leather lining for footwear which contains nanosilver. Silverlining is a system applicable to shoe linings, guaranteeing the breathability of the leather, because the treatment is made with natural products (including caseine) that allow the user to have a very comfortable lining. Silver exerts its antibacterial power constantly and therefore it stops the development of bad smells due to the natural sweat of the foot [29, 30].

Koizhaiganova and others applied silver-doped hydroxyapatite (Ag-HA) prepared by the microwave method, as the finishing agent to leather. The antibacterial activity of the leathers were assessed by means of the parallel streak method (qualitative) and test of the percentage of microbial reduction (quantitative). According to the results of these tests, leather treated with Ag-HA containing 2% or more of silver (Ag) showed good antibacterial activity; thus, the authors suggested that Ag doped hydroxyapatite could be used as an antibacterial agent on lining leathers [31].

\section*{Aim of study}

The aim of the research work was to investigate the biological activity of selected biocides applied to tanned leather intended for footwear, leather fancy goods and carpets. The work included the selection of biocidal preparations containing active substances. Determination of doses used for each commercial preparation and preparations prepared for the treatment of leather in finishing processes were based on data provided by the formulations’ manufacturers.

Research work included microbiological tests of leather samples tanned and finished using the same technology, with different antimicrobial protection and without microbiological treatment. Effectiveness assessment of the biostabilization of the leathers and selection of the most beneficial preparations were carried out. The research was carried out as part of the research project POIR.02.03.02.-12-17-0056/17.

\section*{Materials and methods}

For application tests of biocides intended for leather improvement, ready-made commercial biocides and prepared preparations were taken into account. Prepa-
Preparations prepared:
- S27-22 – 1.5% aqueous solution of a commercial biocide (Sanitized T 27-22 Silver) in the form of a dispersion containing silver chloride and titanium dioxide; CAS: 7783-90-6, 13463-67-7
- SH 22-27 – 1.5% aqueous solution of a commercial biocide (Sanitized TH 22-27) in the form of a dispersion containing zinc pyrithione; CAS: 13463-41-7
- ST 99-19 – 1% aqueous solution of a commercial biocide (Sanitized T 99-19) in the form liquid containing dimethyltetradecyl[3-(trimethoxysilyl)propyl]ammonium chloride; CAS: 41591-87-1
- NS 500 – 100% aqueous solution of nanosilver 500 ppm; CAS 7440-22-4
- NS 500 M250 – 50% aqueous solution of a commercial biocide that contains nanosilver 500 ppm & nanocopper 250 ppm; CAS: 7440-22-4, 7440-50-8
- SU – 0.5% aqueous solution of polyhexamethylene biguanide (PHMB); CAS: 91403-50-8, polymer
- ODH – 100% commercial tea tree oil (Melaleuca) – essential oil obtained by distillation of tea tree leaves; CAS: 68647-73-4
- OP – 100% commercial orange oil – orange peel oil extracted cold; CAS: 8000-57-9
- SGP – saturated solution of aluminium potassium sulphate (potassium aluminium sulfate dodecahydrate); CAS: 7784-24-9

Leather samples protected with preparations
Bovine grain leather of chrome tanning in black colour, with a thickness of 1.25 mm, designed for the production of footwear, was selected for biostabilization trials. The leather was intended for the upper components of footwear but had not been pretreated with any biocides during the manufacturing process in a tannery.

Ten samples of bovine leather were tested:
Sample No. 0 – Bovine leather, control sample.
Sample No. 1 – Bovine leather + S 27-22 (silver chloride and titanium dioxide)
Sample No. 2 – Bovine leather + SH 22-27 (zinc pyrithione)
Sample No. 3 – Bovine leather + ST 99-19 (dimethyltetradecyl[3-(trimethoxysilyl)propyl]ammonium chloride)
Sample No. 4 – Bovine leather + NS 500 (nanosilver 500 ppm)
Sample No. 5 – Bovine leather + NS 500 M 250 (nanosilver 500 ppm + nanocopper 250 ppm)
Sample No. 6 – Bovine leather + SU (PHMB)
Sample No. 7 – Bovine leather + ODH (tea tree oil)
Sample No. 8 – Bovine leather + OP (orange oil)
Sample No. 9 – Bovine leather + SGP (aluminium potassium sulphate)

Microorganisms
The microbiological resistance of the improved leather samples was tested on the action of five species of microorganisms selected. The tests were carried out in relation to the pathogenic bacteria and fungi that often inhabit materials in used footwear.

The bacteria included in the studies were Staphylococcus aureus ATCC 25923 (gram positive), Staphylococcus aureus, contributing to bacterial infections of the skin of the feet, causing purulent changes on the skin, and Escherichia coli ATCC 25922 (gram negative), which can colonise the skin and mucous membranes of the mouth and respiratory system, cause urinary tract infections and meningitis in newborns, and is the most common gram negative bacterium causing sepsis [32, 33].

The fungi used were Candida albicans ATCC 90028 (pathogenic yeast, belonging to the etiological factors of foot and nail fungal infections) and filamentous fungi: Aspergillus brasiliensis (niger) ATCC 16404 (pathogenic species, mould, easily inhabiting materials), and Trichophyton mentagrophytes ATCC 18748 (pathogenic fungus, dermatophyte, which is the etiological factor of the mycosis of skin and nails of the feet and hands, difficult to treat) [34-39].

The aqueous solutions of agents prepared, containing active substances, were applied by spraying with a pneumatic gun in a chamber for applying finishing coatings to leather. The pressure applied to the gun was 1 ± 2 bar, and a nozzle of 0.9 ± 1.2 mm was used for spraying the biocide preparations, where the length of the stream was about 15-20 cm. Biocidal preparations were applied on the leather from the flesh-side for all test specimens as the leather was intended for footwear uppers. The sample surface had dimensions of 297 x 420 mm.

The preparations were applied in the amount of 40 ± 80 ml to one side of the sample depending on the type of preparation (nanopreparations: 40 ± 50 ml, PHMB solution: 50 ml, essential oils: 80 ml, other preparations: 60 ml). The formulations were applied in three steps, i.e. in three subsequent sprays, to obtain an even distribution and better penetration of the solutions into the leather. After each spray of the preparation onto the sample, the leather was dried at temperature (20 ± 1) °C, in a tensioned state, for 2 hours. Table 1 specifies the amount of the individual preparations applied to the leather samples.

Methods of microbiological tests
Nine samples of bovine leather after the treatment with preparations containing active substances and a control sample without microbiological treatment underwent microbiological testing in the form of:
- Evaluation of the resistance to bacteria and determination of the antibacterial effect of the antibacterial treatment according to PN-EN ISO 20645: 2006 Textile fabrics – Determination of antibacterial activity – Agar diffusion plate test [40].
- Evaluation of resistance to fungi and determination of the antifungal effect according to the PN-EN 14119: 2005

Table 1. Amount of preparation applied on leather sample.

| Sample number | Preparation | Volume, ml |
|---------------|-------------|------------|
| 1             | S 27-22     | 60         |
| 2             | SH 22-27    | 60         |
| 3             | ST 99-19    | 60         |
| 4             | NS 500      | 50         |
| 5             | NS 500 M 250| 40         |
| 6             | SU          | 50         |
| 7             | ODH         | 80         |
| 8             | OP          | 80         |
| 9             | SGP         | 60         |
Table 2. Requirements recommended by SBL-IPS for leather with microbial activity.

| Method | Parameter |
|--------|-----------|
| Antibacterial activity according to PN-EN ISO 20645:2006 | Inhibition zone [mm]: >1; 1-0; 0; Bacterial growth: no growth, slight growth |
| Antifungal activity according to PN-EN 14119:2005 | Inhibition zone [mm]: ≥0 |

Table 3. Determination of the antibacterial effect of leather samples against S. aureus and E. coli bacteria. Note: L – grain of leather adheres to the agar, M – flesh of leather adheres to the agar, * microcolonies in the growth inhibition zone.

| Sample number | Staphylococcus aureus | Escherichia coli |
|---------------|-----------------------|------------------|
|               | Inhibition zone, mm L/M | Growth L/M | Assessment L/M | Inhibition zone, mm L/M | Growth L/M | Assessment L/M |
| 1             | 0/0                   | moderate      | insufficient effect/limit of efficacy | 0/0                   | moderate      | insufficient effect /good effect |
| 2             | 1*4                   | none          | good effect /good effect | 0/23                  | moderate      | insufficient effect /good effect |
| 3             | 0/0                   | moderate      | insufficient effect /good effect | 0/0                   | moderate      | insufficient effect /good effect |
| 4             | 0/0                   | moderate      | insufficient effect /good effect | 0/0                   | moderate      | insufficient effect /limit of efficacy |
| 5             | 0/1                   | moderate      | insufficient effect /good effect | 0/0                   | moderate      | insufficient effect /good effect |
| 6             | 0/1(2*)               | moderate      | insufficient effect /good effect | 0/1(2*)              | moderate      | insufficient effect /good effect |
| 7             | 0/0                   | slight        | moderate effect /good effect | 0/1                  | slight        | moderate effect /good effect |
| 8             | 0/0                   | none          | good effect /good effect | 0/0                  | moderate      | insufficient effect /limit of efficacy |
| 9             | 0/0                   | moderate      | insufficient effect /good effect | 0/2                  | moderate      | insufficient effect /good effect |

Table 4. Determination of the antifungal effect of leather samples against yeast C. albicans. Note: L – grain of leather adheres to the agar, M – flesh of leather adheres to the agar, * microcolonies in the growth inhibition zone.

| Sample number | Inhibition zone of growth, mm L/M | Growth L/M | Assessment L/M |
|---------------|----------------------------------|-----------|----------------|
| 1             | 0/3                              | moderate/none | insufficient effect /good effect |
| 2             | 0/1                              | moderate/none | insufficient effect /good effect |
| 3             | 2(3^*)/0                         | none/none | good effect /good effect |
| 4             | 0/0                              | moderate/none | insufficient effect /good effect |
| 5             | 0/1                              | none/none | good effect /good effect |
| 6             | 0/3                              | moderate/none | good effect /good effect |
| 7             | 0/2(3*)                          | none/none | good effect /good effect |
| 8             | 0/1*                             | moderate/none | insufficient effect /good effect |
| 9             | 0/4                              | moderate/none | good effect /good effect |

The research methodology was adapted to the type of material tested and to the nature of the growth of the microorganisms selected. The tests were carried out in triplicate for each trial. The working samples were circular, with a diameter of (25 ± 0.5) mm, and tested on both sides. Only unsterilised samples of leather were used. Blind test samples of identical grain leather but without treatment with biocidal preparations were used to control the growth of microorganisms.

Bacterial cultures were applied for 18-20 hours and yeasts for 48 hours at 37 ± 1 °C. After cultivation, microbial growth around and under the samples was observed. The antimicrobial effect was assessed in accordance with the recommendations of the standard [40]:
- **good effect** – no growth;
- **limit of efficacy** – slight growth (no inhibition zone, only some restricted colonies, growth nearly totally suppressed in comparison to the control);
- **insufficient effect** – moderate growth (no inhibition zone; in comparison to the control growth, reduced by half), heavy growth (no inhibition zone; in comparison to the control no growth, reduced or only slightly reduced growth).

Fungal cultures were cultivated at 29 ± 1 °C for 2-3 weeks. During and after the cultivation, the plates were observed with the unaided eye and under a stereoscopic microscope. The fungal growth was evaluated on the basis of observations, according to the scale included in the standard [41]:
- 0 – no visible growth assessed under the microscope (magnification 50x),
- 1 – no visible growth without magnifying devices, clearly visible under the microscope,
- 2 – visible increase without magnifying devices, covering up to 25% of the examination surface,
3 – visible increase without magnifying devices, covering up to 50% of the examination surface,
4 – significant increase, covering more than 50% of the surface tested,
5 – heavy growth covering the surface tested.

Studies on the effectiveness of biostabilization of the leather using biocides were carried out in cooperation with the Microbiologist Team working at Jagiellonian University’s Collegium Medicum in Krakow, Department of Mycology.

## Results and discussion

### Evaluation of improved leather samples

The samples tested were evaluated according to the quality standards recommended and used by the Łukasiewicz Research Network – Institute of Leather Industry (SBL-IPS) in Krakow for the assessment of microbiological properties of technical materials. The basic requirements for materials with a hygienic finish are shown in Table 2.

Detailed results of the tests of the leather samples with a hygienic finish are presented in Tables 3-6.

The evaluation of the antibacterial activity of the leather samples on the flesh side is presented in Figure 1. Most leather samples with microbiological treatment show a good antibacterial effect on the flesh side. In the case of *S. aureus*, only the control sample (without microbiological protection) demonstrated limited efficacy, as well as sample 1, which was protected with preparation S27-22 (Table 3). In the case of *E. coli*, sample 4 (with NS 500 applied) and sample 8 (with OP formulation) demonstrated limited efficacy on the flesh side of the leather (Table 3).

The evaluation of the antifungal activity of the leather samples on the flesh side is presented in Figure 2. With regard to *C. albicans* yeast, only sample 4, i.e. leather treated with the NS 500 preparation, occurred to be inefficiently protected (Table 4). The filamentous fungi were assessed using two methods: B1 and B2 according to PN-EN 14119: 2005. After analysis of the results, it was possible to conclude that a good effect against moulds was demonstrated by sample 2 (containing SH 22-27), sample 5 (containing SH 22-27), and sample 10 (containing S27-22).

### Table 5. Determination of the antifungal effect of leather samples against *A. brasiliensis* mould. Note: L – grain of leather adheres to the agar, M – flesh of leather adheres to the agar, *: shreds growing on the sample from the agar side.

| Sample number | Method B1 | Method B2 |
|---------------|-----------|-----------|
|               | Inhibition zone of growth, mm L/M | Assessment of growth on samples from the air side L/M | Assessment of growth in agar medium under the sample L/M | Inhibition zone of growth, mm L/M | Assessment of growth in agar medium under the sample L/M |
| 1             | 0/0       | 3/0       | 5/5       | 0/0       | 1/0       |
| 2             | 1/7(12)   | 2/0       | 5/0       | 0/25      | 1/0       |
| 3             | 0/0       | 4/2       | 5/5       | 0/0       | 1/5       |
| 4             | 0/0       | 4/2       | 5/5       | 0/0       | 1/5       |
| 5             | 2/2       | 0/0       | 1/2/1     | 0/0       | 1/0       |
| 6             | 0/7       | 1/0       | 5/0       | 0/1(2’)   | 1/0       |
| 7             | >27->32   | 0/0       | 0/0       | 27/1      | 0/0       |
| 8             | 0/0       | 2/0       | 5/4       | 0/0       | 0/1(3)    |
| 9             | 0/0       | 3/0       | 5/3       | 0/2       | 1/4       |

### Table 6. Determination of the antifungal effect of leather samples against *T. mentagrophytes* dermatophyte. Note: L – grain of leather adheres to the agar, M – flesh of leather adheres to the agar, *: the growth of Penicillium or other moulds that hindered the reading and interpretation of results, ** restricted growth inside the zone, *** an ambiguous result in comparison with the evaluation of sample activity from the side of the flesh. The inhibited growth is probably due to other reasons, X – it was difficult to determine the zone of *T. mentagrophytes* fungal inhibition (increase in Penicillium or other moulds impeding reading and interpretation of results), z – pollution with moulds.

| Sample number | Method B1 | Method B2 |
|---------------|-----------|-----------|
|               | Inhibition zone of growth, mm L/M | Assessment of growth on samples from the air side L/M | Assessment of growth in agar medium under the sample L/M | Inhibition zone of growth, mm L/M | Assessment of growth in agar medium under the sample L/M |
| 1             | 0(6’)/12 | 0(2’)/0 | 1(0’5’)/Rhizopus | X/12 | X/42 |
| 2             | 0(6’)/7  | 0(2’)/0(2’’)/ | 1(0’5’)/Rhizopus | 0/5’ | 2/52 |
| 3             | 0(5’)/11 | 0(2’)/0(2’’) | 3/0(4’)/ | 7/10 | 4/42 |
| 4             | 0(5’)/X  | 0(2’)/0(2’’) | 5/0(4’)/Rhizopus | X’/X’ | 4/0 |
| 5             | 15/15    | 0(0’)/0(2’’) | 4/0 | 17/27 | 4/0 |
| 6             | 7/8      | 0/0(2’’) | 3/0 | 27/27 | 0/0 |
| 7             | 37/37    | 0/0 | 0/0 | 27/27 | 0/0 |
| 8             | 15/28    | 0/0 | 3/0(3’)/ | 27/27 | 0/4z |
| 9             | 5/11     | 0/0 | 4/0(3’)/ | 27/’/12 | 0/2/3z |

### Figure 1. Evaluation of the antibacterial activity of leather samples on the flesh side.
The preparation shows a good antibacterial effect in relation to the bacteria *S. aureus* tested and limited efficiency for *E. coli*, but in both cases inhibition of growth was observed only on the flesh side of the leathers. Moreover, there was insufficient activity against the bacteria on the grain side and on both sides of the leather in relation to yeast *C. albicans*, as average microbial growth was observed. The preparation did not show a biocidal effect on the moulds of *A. brasiliensis* since a plentiful growth of this fungus was observed on the flesh side of the leather. In the case of *T. mentagrophytes* dermatophyte, the result is ambiguous because the samples were intensively overgrown with *Penicillium* mould.

**NS 500** – The preparation has a good antibacterial effect in relation to the bacteria *S. aureus* tested and limited efficiency for *E. coli*, but in both cases inhibition of growth was observed only on the flesh side of the leathers. Moreover, there was insufficient activity against the bacteria on the grain side and on both sides of the leather in relation to yeast *C. albicans*, as average microbial growth was observed. The preparation did not show a biocidal effect on the moulds of *A. brasiliensis* since a plentiful growth of this fungus was observed on the flesh side of the leather. In the case of *T. mentagrophytes* dermatophyte, the result is ambiguous because the samples were intensively overgrown with *Penicillium* mould.

**NS 500 M250** – The preparation has a good antibacterial effect in relation to the bacteria *S. aureus* and *E. coli* tested, but in both cases growth inhibition was observed only on the flesh side of the leather. However, a good effect on yeast *C. albicans* was observed on both sides of the samples. The preparation has a biocidal effect with zones of growth inhibition of mould *A. brasiliensis* and dermatophyte *T. mentagrophytes*. On the grain side, growth of *Penicillium* was observed, due to sample contamination, which means no effective activity against this fungi.

**SU** – The preparation shows a good antibacterial effect on the flesh side of the leather with reference to bacteria *S. aureus* and *E. coli*, and yeast *C. albicans*. However, limited efficacy in the presence of *S. aureus* and an insufficient effect (mean increase) were observed on the grain side of the leather with reference to bacteria. *S. aureus* and *E. colig* test.
Tea tree oil is a preparation that diffuses from the substrate to its surface, giving large zones of microbial inhibition. Therefore, the operation of this preparation should be particularly checked during the storage and use of products made therewith. At the same time, it would be necessary to include in further microbiological tests samples of leathers protected with the preparation directly added to the varnish forming the finishing coat, e.g. for leather intended for the linings with a finish coating. In this case, the operation of this preparation gives a good antibacterial effect and biocidal activity against yeast-like and filamentous fungi with large inhibition zones of growth.

In order to optimise the treatment process of leather, preparations SH 22-27, NS500 M250, ODH and SU should be included in further trials and microbiological tests. It is necessary to check their suitability for leathers with different finishes and purposes (uppers, linings etc.), and also to check their effectiveness during the use of leather products. For this reason, the principles of action of the active substances should be taken into account, as some of them may exhibit high diffusion from the substrate in a short time by evaporation, photodegradation, photooxidation or leaching.

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12. The equipment consists:
   - micrometers (thickness), tensile testing machines (Alwevron), Mullens (bursting strength), Elmendorf (tearing resistance), Bekk, Bendtsen, PPS (smoothness/roughness), Gurley, Bendtsen, Schopper (air permeance), Cobb (water absorptiveness), etc.,
   - crush tester (RCT, CMT, CCT, ECT, FCT), SCT, Taber and Lorentzen&Wettre (bending 2-point method) Lorentzen&Wettre (bending 4-point method) and stiffness resonance method), Scott-Bond (internal bond strength), etc.,
   - IGT (printing properties) and L&W Elrepho (optical properties), etc.,
   - power-driven press, fall apparatus, inline plane tester, vibration table (specialized equipment for testing strength transport packages),
   - atomic absorption spectrometer for the determination of trace element content, pH-meter, spectrophotometer UV-Vis.

Contact:
INSTITUTE OF BIOPOLYMERS AND CHEMICAL FIBRES
ul. M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland
Elżbieta Baranek Dr eng. mech., tel. (+48 42) 638 03 31, e-mail: elobaranek@ibwch.lodz.pl