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THE EVALUATION OF MICROBIAL CONTAMINATION IN THE WORKING ENVIRONMENT OF TANNERIES

OCENA ZANIECZYSZCZENIA MIKROBIOLOGICZNEGO NA STANOWISKACH PRACY W GARBARNIACH

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ABSTRACT

Background: Due to their animal material processing, tannery workers may be exposed to biological agents. The aim of the study was the microbial contamination assessment of tanneries with different production specifications. Health risk was estimated based on particle size distribution. Moreover, indicators of microbial contamination of tanneries were selected. **Materials and Methods:** The studies were conducted in 2 types of tanneries – processing raw hides and producing chrome-tanned leather. Air was sampled with MAS-100 Eco Air Sampler, leathers using RODAC Envirocheck® contact plates and swab method, microbial numbers were determined by a culture method. For the bioaerosols size distribution analysis, a six-stage Andersen sampler was used; identification was performed using microscopy and biochemical methods. Microbial contamination was identified by 16S RNA and ITS1/2 rDNA analysis for bacteria and fungi respectively. **Results:** The microbial number in the air ranged between 1.2×10^3 and 3.7×10^3 CFU/m³. While on the leather, it ranged between 7.6×10^1 and 5.5×10^5 CFU/100 cm². Bacteria dominated in the tanneries (air: 51–92%, leathers: 60–100%). Results indicate that potential health risks arise from the fungal small bioaerosol particles presence (0.65–2.1 μm). Eleven indicator microorganisms were determined: *B. pumilus*, *B. subtilis*, *B. cereus*, *C. lubricantis*, *C. cladosporioides*, *P. commune*, *P. echinulatum*, *P. chrysogenum*, *P. crustosum*, *C. parapsilosis* and *C. albidus*. **Conclusions:** Microbial contamination evaluation in the tanneries showed the increased bacteria and fungi number in the air in relation to the outdoor air, which indicates an occupational inhalation risk to workers. The designated indicators of microbial contamination in the tanneries are associated with their specific and potentially pathogenic working environment. Med Pr 2014;65(1):15–32

Key words: tanneries, bioaerosols, microbial contamination, microbial indicators

STRESZCZENIE

Wstęp: Z powodu przetwarzania surowca zwierzęcego w garbarniach pracownicy mogą być narażeni na czynniki biologiczne. Celem badań była ocena zanieczyszczenia mikrobiologicznego w garbarniach o różnej specyfice produkcji. Określono także ryzyko zdrowotne w oparciu o rozkład ziarninowy bioaerozolu. Ponadto wyznaczono wskaźniki zanieczyszczenia mikrobiologicznego w garbarniach. **Materiał i metody:** Badania przeprowadzono w dwóch rodzajach garbarni – przetwarzających skóry surowe i chromowo garbowane (wet blue). Powietrze pobierano próbnikiem MAS-100 Eco, próby ze skór, używając płytek odciskowych RODAC Envirocheck® i metody tamponowej, a liczbę mikroorganizmów określano metodą hodowlaną. Rozkład cząstek bioaerozolu wykonano z użyciem 6-stopniowego impaktora Andersena. Identyfikację drobnoustrojów wykonano metodą mikroskopową i testami biochemicznymi. Wskaźniki zanieczyszczenia mikrobiologicznego identyfikowano, analizując odpowiednio dla bakterii i grzybów sekwencje 16S RNA i ITS1/2 rDNA. **Wyniki:** Liczebność mikroorganizmów w powietrzu w garbarniach kształtowała się w granicach $1,2 \times 10^3$ – $3,7 \times 10^3$ jtk/m³. Skóry były zanieczyszczone mikrobiologicznie w granicach $7,6 \times 10^1$ – $5,5 \times 10^5$ jtk/100 cm². W garbarniach dominowały liczbowo bakterie (w powietrzu: 51–92%, na skórkach: 60–100%). Wskazano na zagrożenie zdrowotne wynikające z obecności cząstek bioaerozolu grzybowego o rozmiarach 0,65–2,1 μm. Wyznaczono 11 gatunków drobnoustrojów wskaźnikowych dla garbarni: *B. pumilus*, *B. subtilis*, *B. cereus*, *C. lubricantis*, *C. cladosporioides*, *P. commune*, *P. echinulatum*, *P. chrysogenum*, *P. crustosum*, *C. parapsilosis* i *C. albidus*. **Wnioski:** Ocena zanieczyszczenia mikrobiologicznego w garbarniach wykazała podwyższoną liczebność bakterii i grzybów w powietrzu w stosunku do powietrza atmosferycznego, co świadczy o występowaniu narażenia inhalacyjnego pracowników. Wyznaczone wskaźniki zanieczyszczenia mikrobiologicznego w garbarni są związane ze specyfiką środowiska pracy i potencjalnie chorobotwórcze. Med. Pr. 2014;65(1):15–32

Słowa kluczowe: garbarnia, bioaerozol, zanieczyszczenie mikrobiologiczne, wskaźniki mikrobiologiczne

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INTRODUCTION

Leather is an important raw material used in the manufacture of footwear, apparel, furniture and leather products. In 2011, the total turnover of the European Union tanning industry reached 7.8 billion euro, corresponding to the production of 224 million m² of finished leather and about 44 thousand tons of sole leather. Currently, it is estimated that leather product industries in the European Union have about 24 000 of different-sized enterprises employing a total of 400 000 workers (1).

In order to give the leather adequate durability and aesthetic quality, it is necessary to perform a series of mechanical and chemical operations, wet and finishing processes (2,3). These treatments are necessary because fresh animal skins are of limited value, as they contain large amounts of moist and available nutrients (carbohydrates, fats and proteins) that provide a perfect medium for rapid growth of microorganisms (4). The sources of microorganisms are diverse. The skin may be contaminated with blood, feces or soil; the means of transport, air and equipment in production and storage facilities should be also considered. Bacteria that are most often isolated from raw hides belong to the species: *Escherichia coli*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Morganella morganii*, *P. morgani*, *P. vulgaris*, *Bacillus anthracis*, *B. subtilis* and *B. mycoides* (4,5). The action of bacterial proteolytic enzymes results in partial or complete hydrolysis of leather components, the emission of ammonia and hydrogen sulphide, stains and damage to the leathers surface (6,7).

Leather has a higher biological resistance than raw hide. This is because it contains collagen, which is resistant to proteolytic enzymes (except collagenase) and a small amount of water (preferably due to the functional characteristics 14–20%) (8). Microbiological destruction of chrome-tanned leather (wet blue) and leather results mainly from the activity of filamentous fungi (9). The studies of microbial contamination of leather have shown that the dominant species are filamentous fungi from the genera *Penicillium* (*P. commune*, *P. glaucum*, *P. wortmannii*, *P. frequentans*) and *Aspergillus* (*A. niger*, *A. flavus*, *A. oryzae*, *A. fumigatus*). Apart from those, the occurrence of the species from the genera: *Alternaria*, *Cladosporium*, *Trichoderma*, *Fusarium*, *Aureobasidium*, *Scopulariopsis* and also actinomycetes from the genus *Streptomyces* was observed on the surface

of tanned hides (10). They can cause a breakdown of fat and loosening of hide fibres, and create differently coloured stains (11,12). The growth of microorganisms in raw hides, chrome-tanned leather and finished products may pose a threat to tanners, especially those involved in the first operations of tanning. Potential microbiological hazards in the working environment of tanneries may result from the exposure to high concentrations of pathogens in raw material and in the air of production facilities as well as from the contact with pathogens. Pathogenic microorganisms found in the working environment include: *Bacillus anthracis* (anthrax), *Leptospira interrogans* (leptospirosis), *Clostridium tetani* (tetanus), *Coxiella* (Q fever) and *Brucella melitensis*, *B. suis*, *B. abortus* and *B. canis* (brucellosis) (13).

There have been reports concerning various health problems among workers in tanneries. In the study carried out by Ory et al. (14) the health complaints among 418 labourers in 15 Indian tanneries were tested. Asthma (38%), dermatitis (23%) and chronic bronchitis (14%) were the most frequently reported complaints in the 12 months prior to the survey. Moreover, Cr⁶⁺ ions cause skin irritation, eardrum perforation, nasal irritation, ulceration and lung carcinoma in humans and animals, and are accumulated in placenta impairing the foetal development in mammals (14). Rastogi et al. (15) analysed the health risks of 197 male workers drawn from different sections of 10 leather tanneries in Kanpur. A control group comprising of 117 male subjects belonging to a similar age group and socioeconomic strata, who never had any occupational exposure in the leather tanneries, were also examined for comparison. The findings revealed a significantly higher prevalence of morbidity among the exposed workers in contrast to that observed in the controls (40.1% vs. 19.6%).

Respiratory diseases (16.7%) were mainly responsible for a higher morbidity among the exposed workers, whereas gastrointestinal tract problems were predominant in the control group (15). There has only been a limited number of studies on the microbiological risks in the working environment of tanneries. The aim of the present studies was to analyse the degree of microbial contamination and the type of microorganisms in the working environment of tanneries with different production specifications – those processing raw hides and those producing wet blue leather. Health risk was also estimated basing on a study of particle size distribution in a selected facility.

Moreover, indicator microorganisms were selected for the working environments of the tanneries basing on an analysis of their prevalence in a workplace, source of isolation, and health risk determined based on literature data (according to the classification of the Directive 2000/54/EC (16)), the Regulation of the Minister of Health in Poland dated 22nd of April, 2005 (17), the biosafety levels classification (BSL) of the European Confederation of Medical Mycology (18) and the Institute of Rural Health in Lublin (Instytut Medycyny Wsi im. Witolda Chodźki w Lublinie) (13). The taxonomic classification of the selected indicators was confirmed using molecular methods.

MATERIAL AND METHODS

Description of the studied rooms

The assessment of microbial contamination was performed in 4 tanneries with different production specifications. Two of the studied tanneries (Tanneries I and II) produced wet blue leather, and the production process was mainly based on retanning, dyeing and finishing leather. The 2 other plants (Tanneries III and IV) specialized in the production of leather for shoes and fancy goods; the production process included all stages of raw hide (fresh and salted) processing and ended up obtaining leather for the production of leather goods.

Description of the tested rooms is given in Table 1. Temperature and humidity in the tested rooms were determined using a PWT-401 hygrometer (Elmetron, Poland). Samples of the air were also taken in an office room (internal background) and in the atmospheric air (external background).

Determination of microbiological contamination of the air and leathers

Samples for the analysis of microbial contamination of the air and leather surface in the plants were collected during active working hours. Microbiological contamination of the air was determined using an MAS-100 Eco Air Sampler (Merck, Germany). Samples of 50 l and 100 l of air, with flow rate: 100 l/min, were taken directly onto MEA (Malt Extract Agar, Merck, Germany) with chloramphenicol (0.1%) for determination of the total number of fungi (including hydrophilic and xerophilic fungi), and onto a TSA medium (Tryptic Soy Agar, Merck, Germany) with nystatin (0.2%) for the determination of the total number of bacteria. Air samples were taken in 2 repetitions on each medium in 3 places in each room.

Table 1. Characteristics of the rooms examined in tanneries
Tabela 1. Charakterystyka pomieszczeń w badanych garbarniach

Tested plant Badany zakład	Rooms Pomieszczenia	Cubature Kubatura [m ³]	Temperature of the air Temperatura powietrza [°C]		Relative humidity of the air Wilgotność względna powietrza [%]		Rooms' destination Przeznaczenie pomieszczenia	Place of sampling Miejsce poboru prób
			M	SD	M	SD		
Tannery I / Garbarnia I	production halls / hale produkcyjne (N = 3)	250–560	26.1	4.71	59.8	10.5	retannage and finishing of wet blue leather, short-term storage of palettes of raw material, vacuum drying of hides, movement of hides using hoists / dogarbowanie i wykańczanie skór wet blue, krótkoterminowe przechowywanie palet ze skórami surowymi, próżniowe suszenie skór, transportowanie skór za pomocą wciągarki	samples were collected next to the palette of wet blue leathers, next to the vacuum drying oven, next to the racks with dried leather, in the tanned leather warehouse / próbki pobrano przy paletcie ze skórami wet blue przy suszarni próżniowej, przy wyciągu z wysuszonymi skórami, w magazynie skór garbowanych
Tannery II / Garbarnia II	production halls / hale produkcyjne (N = 3)	370–650	15.9	1.1	48.1	6.1	retannage of wet blue leather, sorting, shaving, fluffing, drying and cutting, finishing of leather, short-term storage of hides / dogarbowanie skór wet blue, sortowanie, odwłascianie, szlifowanie, suszenie i cięcie oraz wykończenie skór, krótkoterminowe przechowywanie skór	samples were taken next to the planing machine, next to the tanning drum, next to the sanding and stretching machine, over the tanning racks, next to the leather cutting machine, next to the racks with tanned leather / próbki pobrano przy strugarce przy bębnie garbarskim przy szlifierce z napinarką nad wieszakami ze skórami, przy urządzeniu do cięcia skór przy wieszakach z skórą garbowaną

Table 1. Characteristics of the rooms examined in tanneries – cont.
Tabela 1. Charakterystyka pomieszczeń w badanych garbarniach – cd.

Tested plant Badany zakład	Rooms Pomieszczenia	Cubature Kubatura [m ³]	Temperature of the air		Relative humidity of the air		Rooms' destination Przeznaczenie pomieszczenia	Place of sampling Miejsce poboru prób
			Temperatura powietrza [°C]	SD	Wilgotność względna powietrza [%]	SD		
Tannery III / Garbarnia III	production halls / hale produkcyjne (N = 2)	300–18 000	16.4	0.8	39.2	4.9	movement and segregation of raw material, soaking, liming and shaving of raw hides, tanning and dyeing hides. Samples were taken next to the soaking and liming drums, next to the pallet of raw hide scraps / transportowanie i segregacja skór surowych, moczenie, wapnowanie i odwiązanie skór surowych, garbowanie i farbowanie skór. Próby pobrano przy bębnoch do moczenia i wapnienia, przy paletce ze ścinkami świeżej skóry	samples were collected next to the racks with sorted wet blue leathers, next to the planing machine, next to the dyeing drums / próbki pobrano przy wieszakach z posortowanymi skórami wet blue, przy strugarce, przy bębnoch do barwienia skór
Tannery IV / Garbarnia IV	warehouses for raw, wet blue and tanned leath- ers, produc- tion halls / magazyny skór surow- ych, wet blue i gar- bowanych, hale produkcyjne (N = 6)	285–27 000	20.2	4.4	50.8	17.7	storage of salted raw hides, preliminary and main soaking, rinsing of hides, liming, bating, pickling, cutting of wet blue leathers, storage of wet blue and tanned leathers / przechowywanie solonych skór surowych, moczenie wstępne i główne, płukanie skór surowych, odmięśnianie, wytrawianie, piklowanie, cięcie skór wet blue, przechowywanie skór wet blue i garbowanych	samples were taken in the raw hide warehouse, next to the pallets of raw hides after adding salt, next to the pre-soaking drum, next to the soaking drum, next to the pallets of skins after soaking, next to the rinsing drum, next to the fleshing machine, next to the liming drum, next to the pallets of hides immediately after liming, next to the pallets of hides after bathing, next to the pallets of hides after pickling, next to the cutting machine for wet blue hides, next to the racks with tanned leather / próby pobrano w magazynie skór surowych, przy paletach ze skórami po dodaniu soli, przy bębnie do moczenia wstępnego, przy bębnie do moczenia właściwego, obok palet ze skórami po etapie moczenia, obok palet ze skórami po moczeniu właściwym, przy bębnie do płukania, przy urządzeniu do odmięśniania skór, przy bębnie do wapnienia, obok palet ze skórami zaraz po wapnieniu, obok palet ze skórami po wytrawianiu, obok palet ze skórami po piklowaniu, przy urządzeniu do cięcia skór wet blue, przy wieszakach ze skórą garbowaną
Internal background / Tło wewnętrzne	offices / biura (N = 4)	75–160	17.6	0.6	36.6	4.3	non-productive rooms of tanneries, located in separate buildings, and having a standard office equipment / pomieszczenia w zakładach niezwiązane z produkcją, ulokowane w oddzielnych budynkach i posiadające standardowe wyposażenie biurowe	samples were taken in 3 representative places from each office / próby pobierano w 3 reprezentatywnych miejscach każdego biura
External background / Tło zewnętrzne	atmospheric air / powietrze atmosferyczne (N = 4)	n.t.	n.t.	n.t.	n.t.	n.t.	samples were taken at a distance of 5–10 km from each tannery / próby pobierano w odległości 5–10 km od każdej garbarni	

M – mean / średnia; SD – standard deviation / odchylenie standardowe.
n.t. – not tested / nie badano.

For the analysis of the size distribution of viable bioaerosols, a six-stage Andersen sampler (model WES-710, Westech Instruments, UK) was used. The use of this instrument made it possible to divide the bioaerosols into 6 fractions, in accordance with their aerodynamic diameters, as follows: $\geq 7.0 \mu\text{m}$ (1st stage), $7.0\text{--}4.7 \mu\text{m}$ (2nd), $4.7\text{--}3.3 \mu\text{m}$ (3rd), $3.3\text{--}2.1 \mu\text{m}$ (4th), $2.1\text{--}1.1 \mu\text{m}$ (5th) and $1.1\text{--}0.65 \mu\text{m}$ (6th). The air was sampled with a vacuum pump at a constant 28.3 l/min flow rate. Samples were taken over 5 min (141.5 l of air) onto MEA with chloramphenicol (0.1%) (fungi) or TSA medium with nystatin (0.2%) (bacteria) in 3 repetitions on each medium in the production hall in Tannery I.

The materials processed in tanneries were also analyzed for microbiological content. The names of leather have been adapted from Thanikaivelan et al. (2004) (3):

- hide – skin of a large animal, such as cow or buffalo,
- leather – a general term for hide or skin with its original fibrous structure more or less intact, and that has been treated so as to be imputrescible, the hair or wool might or might not have been removed,
- wet blue – chrome-tanned leather in wet condition.

Samples from leathers were taken using RODAC Envirocheck® plates (Replicate Organism Detection And Counting, Merck, Germany) with TSA medium for bacteria and with Sabouraud medium (Merck, Germany) for fungi. For the analysis of the microbial contamination of hides, a traditional swab method was applied, using saline solution (0.85% NaCl), swabs and metal frames of surface area 25 cm^2 and media: MEA with chloramphenicol (0.1%) (fungi) or TSA medium with nystatin (0.2%) (bacteria). Samples were taken from 3 surfaces on each medium.

The media samples from the air and leather materials were incubated at $30 \pm 2^\circ\text{C}$ for 48 h (bacteria) or at $27 \pm 2^\circ\text{C}$ for 5–7 days (fungi). After incubation, colonies were counted, and the results were expressed in CFU/ m^3 air, CFU/ 100 cm^2 materials. The final result was the arithmetic mean of all of the repetitions.

Identification of microorganisms

The pure cultures of bacteria and yeast were characterized macroscopically, and then, selected diagnostic features were tested: Gram-staining, catalase test and oxidase test (Microbiologie Bactident Oxydase, Merck, Germany). For isolates of bacteria whose frequency of occurrence was greater than 25%, API tests were performed (BioMérieux, France): API 50 CH, API STAPH and API 20 NE, for yeasts, diagnostics was performed using the API C AUX test. Bacteria identi-

fied as indicators of microbiological contamination at workplaces in tanneries were determined according to the procedure described below, underwent the genetic identification based on the nucleotide sequence of gene 16S rRNA (19).

Identification of all of the isolated filamentous fungi was performed basing on macroscopic and microscopic observations after culture on CYA medium (Difco, USA) and YES medium (Yeast Extract with Supplements), with the use of a taxonomic method (17–23).

Identification of moulds and yeast which were specified as the indicators of microbial contamination at workplaces was performed basing on the ITS1/2 sequence of the rDNA region (24). Genomic DNAs of indicators strains were extracted using the described method (25). The resulting nucleotide sequences of the studied microorganisms were analysed and compared with the sequences published in the National Center for Biotechnology Information (NCBI) database, using the BLASTN 2.2.27+ program (26). The sequences obtained for microorganisms were deposited in the NCBI GenBank database.

Selection of indicators of microbiological contamination at workplaces

In order to determine the indicators of microbiological contamination at workplaces in tanneries, protocols or characteristics were established according to Skóra et al. (2012) (27) and modified (frequency of strains isolation, source of isolation and harmfulness to human health) as given in Table 2. A scale of evaluation was also developed for identifying indicators of microbiological contamination at workplaces as given in Table 3.

Mathematical calculations

The frequency of occurrence of a species (f) was calculated by dividing the number of samples in which the strain occurred and the total number of samples.

Statistical analyses were performed using STATISTICA 6.0 software (Statsoft, USA). The obtained results of microbiological contamination of the air and leathers in tanneries with various production profile were evaluated using one-way Analysis of Variance (ANOVA) at the significance level 0.05. When statistical difference was detected ($p < 0.05$), means were compared by the *post-hoc* Tukey's test at significance level 0.05.

Linear regression analysis was used to determine the effect of air humidity and air temperature on microbiological contamination of the air in tested tanneries.

Table 2. Criteria for selecting indicators of contamination with harmful biological agents at tanneries*
Tabela 2. Kryteria typowania wskaźników zanieczyszczenia szkodliwymi czynnikami biologicznymi na stanowiskach pracy*

No. Lp.	Criteria Kryterium	Description Opis	Scale Skala	Points Punkty
1	frequency of strain isolation from the environment / częstość izolacji szczepu ze środowiska	the frequency [%] with which the organism was isolated from the air and/or surface at the workstations / częstość [%], z jaką izolowano dany drobnoustroj z powietrza i/lub powierzchni w pomieszczeniach na stanowiskach pracy	0–20% 21–40% 41–60% 61–80% 81–100%	1 2 3 4 5
2	strain isolation source / źródło pochodzenia szczepu	the presence of microorganism in indoor air and/or on the surfaces at workstations with regard to its presence in the outdoor air (background) / występowanie mikroorganizmu, w powietrzu wewnętrznym i/lub na powierzchniach, na stanowiskach pracy w odniesieniu do jego występowania w powietrzu atmosferycznym (tło)	presence in the indoor air and outdoor air, absence on surfaces / obecność w powietrzu wewnętrznym oraz w powietrzu atmosferycznym, brak na powierzchniach presence on surfaces and outdoor air, absence in the indoor air / obecność na powierzchniach oraz w powietrzu atmosferycznym, brak w powietrzu wewnętrznym presence in the indoor air, absence in the outdoor air and on surfaces / obecność w powietrzu wewnętrznym, brak w powietrzu atmosferycznym i na powierzchniach presence on surfaces, absence in the indoor air and outdoor air / obecność na powierzchniach, brak w powietrzu atmosferycznym i wewnętrznym	1 2 3 4
			presence in the indoor air, outdoor air and on surfaces / obecność w powietrzu wewnętrznym, powietrzu atmosferycznym i na powierzchniach presence in the indoor air and on surfaces, absence in the outdoor air / obecność w powietrzu wewnętrznym i na powierzchniach, brak w powietrzu atmosferycznym	5 6
3	harmfulness to human health / szkodliwość zdrowotna dla ludzi**	according to classification: the Directive UE 2000/54/EC (16), the Regulation of the Minister of Health in Poland dated April 22, 2005 (17), biosafety levels of the European Confederation of Medical Mycology (BSL) (18), Institute of Rural Health in Lublin (IMW) classification (13), literature data / wg klasyfikacji przyjętej w Dyrektywie UE 2000/54/WE (16), Rozporządzeniu Ministra Zdrowia z dnia 22 kwietnia 2005 r. (17), poziomów bezpieczeństwa European Confederation of Medical Mycology (BSL) (18), klasyfikacji Instytutu Medycyny Wsi im. Witolda Chodźki w Lublinie (IMW) (13), danych literaturowych	no data on health risks / brak danych na temat szkodliwości zdrowotnej harmfulness of species according to literature / szkodliwość zdrowotna według danych literaturowych class 2 according to IMW classification (13) or class BSL1 (18) or harmfulness according to literature and confirmed in laboratory studies on strain isolated from work environment / klasa 2 wg klasyfikacji IMW (13) lub klasa BSL1 (18), albo szkodliwość wynikająca z danych literaturowych i potwierdzona w badaniach laboratoryjnych dla szczepu wyizolowanego ze środowiska pracy*** class 2 according to Directive UE 2000/54/EC (16) or class 2 according to the Regulation of the Minister of Health dated April 22, 2005 (17) or class BSL2 (18) / klasa 2 wg Dyrektywy UE 2000/54/WE (16) lub klasa 2 wg Rozporządzenia Ministra Zdrowia z dnia 22 kwietnia 2005 r. (17), lub klasa BSL2 (18) class 3 according to Directive UE 2000/54/EC (16) or class 3 according to the Regulation of the Minister of Health dated April 22, 2005 (17) or class BSL 3 (18) or higher classes / klasa 3 wg Dyrektywy UE 2000/54/WE (16) lub klasa 3 wg Rozporządzenia Ministra Zdrowia (17), lub klasy wyższe	1 2 3 4 5

* Based on / Na podstawie: Skóra et al. Harmful biological agents at museum workstations (27).

** Necessary for belonging to a III group of indicators is obtaining ≥ 3 point from criteria: harmfulness to human health / Niezbędne do zakwalifikowania do III grupy wskaźników jest uzyskanie ≥ 3 punktów z kryterium: szkodliwość zdrowotna dla ludzi.

*** Production of mycotoxins, allergens, exotoxins, haemolysins, polysaccharide capsules, agglutinins and other virulence factors depending on the species / Wytwarzanie mykotoksyn, alergenów, egzotoksyn, hemolizyn, otoczek, aglutynin i innych czynników wirulencji w zależności od gatunku.

BSL1 – saprophytes or plant pathogens occupying non vertebrate ecological niches or fungi utilizing dead animal products. Infections are coincidental, superficial and non-invasive or mild / saprofity lub patogeny roślin zajmujące nisze ekologiczne bezkręgowców lub grzyby wykorzystujące produkty rozkładu martwych zwierząt. Zakażenia są powierzchniowe, nieinwazyjne lub łagodne.

BSL2 – species principally occupying nonvertebrate ecological niches, but with a relatively pronounced ability to survive in vertebrate tissue. In severely immunocompromised humans, they may cause deep, opportunistic mycoses. Pathogens causing superficial infections also fall into this category / gatunki zajmujące nisze ekologiczne bezkręgowców, ale z relatywnie dużą zdolnością do przetrwania w tkankach kręgowców. U ludzi z obniżoną odpornością mogą wywoływać głębokie zakażenia. Do tej kategorii należą także patogeny powodujące zakażenia powierzchniowe.

BSL3 – pathogens potentially able to cause severe, deep mycoses in healthy humans / patogeny potencjalnie zdolne do wywoływania poważnych głębokich grzybic u zdrowych ludzi.

Table 3. The evaluation scale for selecting indicators of microbial contamination at the examined tanneries' workstations
Tabela 3. Skala do wyznaczania wskaźników zanieczyszczenia mikrobiologicznego na stanowiskach pracy w badanych garbarniach

Group of indicators Grupa wskaźników	Points Punkty	Evaluation Ocena	Description Opis
I	3–6	microorganisms not associated with the specificity of tanneries / mikroorganizmy niezwiązane ze specyfiką garbarni	microorganisms occurring in the outdoor air and occasionally in rooms with low microbiological contamination, not constituting health hazard to workers / mikroorganizmy występujące w powietrzu atmosferycznym i sporadycznie w pomieszczeniach o niskim stopniu zanieczyszczenia mikrobiologicznego, niestanowiące zagrożenia zdrowotnego dla pracowników
II	7–10	microorganisms constantly present in the tanneries / mikroorganizmy stale obecne w garbarniach	microorganisms occurring in the indoor air or on surfaces in the rooms with low microbiological contamination, may also be present in the outdoor air, not constituting health hazard to workers / mikroorganizmy występujące w powietrzu lub na powierzchniach w muzeach o niskim stopniu zanieczyszczenia mikrobiologicznego, mogą występować również w powietrzu atmosferycznym, niestanowiąc zagrożenia / stanowiąc zagrożenie zdrowotne dla pracowników
III*	11–16	indicators of microbial contamination at workplaces in tanneries / wskaźniki zanieczyszczenia szkodliwymi czynnikami biologicznymi na stanowiskach pracy w garbarniach	microorganisms occurring with high frequency in areas of high microbial contamination, both in the air and on surfaces, occasionally also occurring in the outdoor air, constituting a health hazard to workers / mikroorganizmy występujące z dużą częstością w pomieszczeniach silnie zanieczyszczonych mikrobiologicznie zarówno w powietrzu, jak i na powierzchniach, sporadycznie występujące również w powietrzu atmosferycznym, stanowiąc zagrożenie dla zdrowia pracowników

* Necessary for belonging to a III group of indicators is obtaining ≥ 3 point from criteria: harmfulness to human health / Niezbędne do zakwalifikowania do III grupy wskaźników jest uzyskanie ≥ 3 punktów z kryterium: szkodliwość zdrowotna dla ludzi.

RESULTS

The number of microorganisms in the air of the tannery rooms ranged between 1.2×10^3 and 3.7×10^3 CFU/m³. The number of bacteria in the tanneries producing chrome-tanned leather, the so-called wet blue (Tanneries I and II) was in the range from 5.1×10^2 to 6.1×10^2 CFU/m³, while in Tanneries III and IV, where leather production begun from the raw materials stage, it was higher, and reached 2.3×10^3 to 3.3×10^3 CFU/m³ ($p < 0.05$).

Contamination with filamentous fungi in the air of the tanneries remained at a lower level, reaching 3.1×10^2 – 1.4×10^3 CFU/m³ (Table 4).

The prevalence of bacteria in the air was reported in 3 out of 4 tested plants (51–92% of all of the isolated microorganisms). The number of microorganisms detected on leathers ranged from 7.6×10^1 to 5.5×10^5 CFU/100 cm². On the surface of raw hides, the number of bacteria was at the level of 5.5×10^5 to 1.2×10^6 CFU/100 cm², and it was lower on wet blue leathers by about 4 orders of magnitude on the logarithmic scale (from 7.6×10^1 to 5.9×10^2 CFU/100 cm²). Bacteria constituted 60–100% of the total number of microorganisms isolated from test leathers (Table 5).

A qualitative analysis revealed the presence of 80 strains isolated in the tanneries, the highest species diversity was found for filamentous fungi (46 isolates), bacteria (25), and the lowest for yeasts (9).

Bacteria most frequently isolated from the air of the tanneries belong to the genera: *Bacillus*, *Brevibacillus*, *Brevundimonas*, *Corynebacterium*, *Kocuria*, *Micrococcus*, *Staphylococcus*. Bacteria from the genera of *Micrococcus*, *Staphylococcus*, and *Pseudomonas* were the most common bacteria species on leather surfaces (Table 6).

Moulds included *Cladosporium*, and *Penicillium*, and as for yeasts, *Cryptococcus* spp. were most frequently isolated from the leather processing plants. The leather surface was most often inhabited by *Penicillium*, *Cryptococcus*, *Kodamaea* and *Pichia* fungi (Table 6).

Basing on the developed criteria, taking into account the prevalence, source of isolation and health hazard, 11 species of indicator microorganisms for the tanneries were determined (risk group 3 was confirmed in the literature or by laboratory tests conducted by the authors in previous studies): *B. pumilus*, *B. subtilis*, *B. cereus*, *Corynebacterium lubricantis*, *C. cladosporioides*, *Penicillium commune*, *P. echinulatum*, *P. chrysogenum*, *P. crustosum*, *Candida parapsilosis* and *Cryptococcus albidus* (Table 6). The analysis of viable

Table 4. Quantitative analysis of microbial contamination in the air in the examined tanneries
Tabela 4. Analiza ilościowa zanieczyszczenia mikrobiologicznego powietrza w badanych garbarniach

Place of sampling Miejsce poboru prób	Fungi in the air Grzyby w powietrzu [CFU/m ³]			Bacteria in the air Bakterie w powietrzu [CFU/m ³]			Microbes in the air Drobnoustroje w powietrzu		
	M±SD	min.–max	M±SD	M±SD	min.–max	total ogółem [CFU/m ³]	fungi grzyby (N = 6) [%]	bacteria bakterie (N = 6) [%]	min.–max
Tannery I / Garbarnia I (N = 3)	$7.3 \times 10^2 \pm 3.0 \times 10^2$	1.7×10^2 – 2.2×10^3	$5.1 \times 10^3 \pm 2.5 \times 10^3$	$1.2 \times 10^3 \pm 5.4 \times 10^2$	1.5×10^3 – 1.5×10^5	4.1×10^2 – 3.6×10^3	59	41	
Tannery II / Garbarnia II (N = 3)	$5.8 \times 10^2 \pm 3.9 \times 10^2$	2.0×10^1 – 2.0×10^3	$6.1 \times 10^3 \pm 3.0 \times 10^3$	$1.2 \times 10^3 \pm 6.9 \times 10^2$	2.0×10^1 – 2.9×10^3	4.0×10^1 – 4.9×10^3	49	51	
Tannery III / Garbarnia III (N = 2)	$1.4 \times 10^3 \pm 1.9 \times 10^2$	2.0×10^2 – 2.8×10^3	$2.3 \times 10^3 \pm 1.4 \times 10^4$	$3.7 \times 10^3 \pm 4.6 \times 10^2$	3.0×10^2 – 4.0×10^3	5.8×10^2 – 6.1×10^3	37	63	
Tannery IV / Garbarnia IV (N = 6)	$3.1 \times 10^2 \pm 2.1 \times 10^2$	6.0×10^1 – 1.7×10^3	$3.3 \times 10^3 \pm 2.4 \times 10^3$	$3.6 \times 10^3 \pm 2.6 \times 10^3$	2.6×10^2 – 1.1×10^4	3.0×10^2 – 1.2×10^4	8	92	
Internal background / Tło wewnętrzne (N = 4)	$1.6 \times 10^2 \pm 2.0 \times 10^2$	2.5×10^1 – 4.5×10^2	$8.6 \times 10^1 \pm 8.0 \times 10^2$	$1.0 \times 10^2 \pm 9.7 \times 10^2$	1.6×10^2 – 1.6×10^3	1.8×10^2 – 2.0×10^3	14	86	
External background / Tło zewnętrzne (N = 4)	$3.5 \times 10^2 \pm 4.3 \times 10^2$	7.0×10^1 – 9.8×10^2	$5.9 \times 10^1 \pm 4.7 \times 10^2$	$9.4 \times 10^1 \pm 7.8 \times 10^2$	1.7×10^1 – 1.0×10^3	2.4×10^2 – 2.0×10^3	36	64	

min. – minimum value / wartość minimalna; max – maximum value / wartość maksymalna.
Other abbreviations as in Table 1 / Inne skróty jak w tabeli 1.

Table 5. Quantitative analysis of microbial contamination on leathers and hides in the examined tanneries
Tabela 5. Analiza ilościowa zanieczyszczenia mikrobiologicznego skór garbowanych i surowych w badanych garbarniach

Place of sampling Miejsce poboru prób	Fungi on leather/hides Grzyby na skórach garbowanych/surowych [CFU/100 cm ²]		Bacteria on leather/hides Bakterie na skórach garbowanych/surowych [CFU/100 cm ²]		Microbes on leather/hides Drobnoustroje na skórach garbowanych/surowych				
	M±SD	min.-max	M±SD	min.-max	M±SD	total ogółem [CFU/100 cm ²]		fungi grzyby (N = 6) [%]	bacteria bakterie (N = 6) [%]
						min.-max	min.-max		
Tannery I / Garbarnia I (N = 3)	1.5×10 ¹ ±1.5×10 ¹	5.1×10 ⁰ -4.6×10 ¹	6.1×10 ¹ ±6.3×10 ¹	1.0×10 ¹ -2.0×10 ²	7.6×10 ¹ ±5.8×10 ¹	1.5×10 ¹ -2.1×10 ²	20/0	80/0	
Tannery II / Garbarnia II (N = 3)	2.3×10 ² ±2.1×10 ¹	1.2×10 ² -4.5×10 ²	2.3×10 ² ±1.9×10 ²	1.8×10 ² -3.1×10 ²	5.9×10 ² ±1.4×10 ²	4.5×10 ² -7.3×10 ²	39/0	61/0	
Tannery III / Garbarnia III (N = 3)	1.5×10 ⁷ ±1.3×10 ⁷ /0	5.6×10 ⁷ /0-2.4×10 ⁷ /0	2.3×10 ⁷ /1.2×10 ⁶ ± ±5.4×10 ¹ /3.0×10 ⁵	1.9×10 ⁷ /8.6×10 ⁵ - -2.6×10 ⁷ /1.6×10 ⁶	3.7×10 ⁷ /1.2×10 ³ ± ±7.6×10 ¹ /3.0×10 ⁵	3.2×10 ⁷ /8.6×10 ⁵ - -4.3×10 ⁷ /1.6×10 ⁶	40/0	60/100	
Tannery IV / Garbarnia IV (N = 3)	1.0×10 ¹ /0±5.1×10 ⁰ /0	5.1×10 ⁰ /0-1.5×10 ¹ /0	2.9×10 ⁷ /5.5×10 ⁵ ± ±2.0×10 ² /3.7×10 ³	5.6×10 ¹ /5.0×10 ⁵ - -4.4×10 ² /5.8×10 ⁵	3.0×10 ⁷ /5.5×10 ⁵ ± ±2.1×10 ² /3.7×10 ⁴	6.1×10 ¹ /5.0×10 ⁵ - -4.5×10 ² /5.8×10 ⁵	3/0	97/100	

Abbreviations as in Tables 1 and 4 / Inne skróty jak w tabeli 1 i 4.

Table 6. Classification of microorganisms from the work environment in tanneries into groups of indicators of microbial contamination
Tabela 6. Mikroorganizmy środowiska pracy w garbarniach vs grupy wskaźników zanieczyszczenia mikrobiologicznego

Microorganism Mikroorganizm	Frequency of occurrence in all of the samples in tanneries (air/surfaces) Częstość występowania we wszystkich próbach w garbarniach (powietrze/powierzchnie) [%]		Presence in the outdoor air Obecność w powietrzu zewnątrzym		Indicators of microbial contamination in tanneries Wskaźniki zanieczyszczenia mikrobiologicznego w garbarniach		
	A	B	isolation frequency częstość izolacji	source źródło	harmfulness to human szkodliwość dla ludzi	total ogółem	group of indicators grupa wskaźników
<i>Bacillus cereus</i>	0/0	0/67 ¹	-	4	3 ^a	11	III*
<i>Bacillus licheniformis</i>	0/60 ¹	0/0	+	3	1	6	I
<i>Bacillus mycoides</i>	0/30 ¹	79/0	-	4	1	11	III
<i>Bacillus pumilus</i>	0/67 ¹	57/0	-	4	3	13	III*
<i>Bacillus</i> sp.	67/0	33/0	+	4	1	6	I
<i>Bacillus subtilis</i>	27/60 ¹	0/0	-	3	6	12	III*
<i>Brevibacillus</i> sp.	100/0	0/0	-	5	3	9	II

Table 6. Classification of microorganisms from the work environment in tanneries into groups of indicators of microbial contamination – cont.
Tabela 6. Mikroorganizmy środowiska pracy w garbarniach vs grupy wskaźników zanieczyszczenia mikrobiologicznego – cd.

Microorganism Mikroorganizm	Frequency of occurrence in all of the samples in tanneries (air/surfaces) Częstość występowania we wszystkich próbach w garbarniach (powietrze/powierzchnie) [%]		Presence in the outdoor air Obecność w powietrzu zewnątrznym	Indicators of microbial contamination in tanneries Wskaźniki zanieczyszczenia mikrobiologicznego w garbarniach				total ogółem	group of indicators grupa wskaźników
	A	B		isolation frequency częstość izolacji	source źródło	harmfulness to human szkodliwość dla ludzi			
<i>Corynebacterium accolens</i> *	100/0	0/0	-	5	3	3	11	III*	
<i>Corynebacterium propinquum</i>	0/0	79/0	-	4	3	3	10	II	
<i>Kocuria rosea</i>	23/0	79/67 ¹	+	4	5	1	10	II	
<i>Kocuria varians</i>	100/0	40/0	+	5	1	1	7	II	
<i>Micrococcus luteus</i>	0/0	93/0	+	5	1	1	7	II	
<i>Micrococcus lylae</i>	0/0	100/100 ¹	+	5	5	1	11	III	
<i>Micrococcus</i> sp.	81/100 ¹	43/0	+	5	5	1	11	III	
<i>Paenibacillus polymyxa</i>	31/0	0/0	-	2	3	1	6	I	
<i>Pseudomonas alcaligenes</i>	0/0	33/0	-	2	3	3	8	II	
<i>Pseudomonas fluorescens</i>	0/0	57/0	-	3	3	3	9	II	
<i>Pseudomonas luteola</i>	0/0	0/100 ^h	+	5	2	3	10	II	
<i>Pseudomonas putida</i>	0/0	0/100 ^h	+	5	2	3	10	II	
<i>Staphylococcus cohnii</i>	0/0	71/0	+	4	1	1	6	I	
<i>Staphylococcus hominis</i>	95/30 ¹	0/0	+	5	5	2	12	III	
<i>Staphylococcus lentus</i>	50/0	86/0	+	5	1	1	7	II	
<i>Staphylococcus sciuri</i>	0/60 ¹	0/100 ¹	+	5	2	1	8	II	
<i>Stenotrophomonas maltophilia</i>	22/0	0/0	-	2	3	1	6	I	
Filamentous fungi / Pleśnie									
<i>Acremonium</i> sp.	33/0	0/0	+	2	1	1	4	I	
<i>Alternaria alternata</i>	39/0	0/0	+	2	1	3	6	I	
<i>Aspergillus clavatus</i>	11/0	0/0	-	1	3	3	7	II	
<i>Aspergillus flavus</i>	4/0	7/0	-	1	3	4	8	II	
<i>Aspergillus niger</i>	15/0	0/0	+	1	1	3	5	I	
<i>Aspergillus sydowii</i>	19/0	0/0	-	1	3	3	7	II	

<i>Aspergillus ustus</i>	0/0	2/0	-	1	3	3	7	II
<i>Aspergillus versicolor</i>	0/0	15/0	-	1	3	3	7	II
<i>Aureobasidium pullulans</i>	15/0	0/0	-	1	3	3	7	II
<i>Beauveria</i> sp.	0/0	15/0	-	1	3	1	5	I
<i>Botrytis cinerea</i>	22/0	24/0	+	2	1	1	4	I
<i>Chaetomium globosum</i>	0/0	0/50 ^l	-	3	4	3	10	II
<i>Cladosporium cladosporioides</i>	83/10 ^l	79/0	+	5	5	3	13	III*
<i>Cladosporium herbarum</i>	100/0	2/0	+	5	1	3	9	II
<i>Cladosporium macrocarpum</i>	78/0	78/0	+	4	1	1	6	I
<i>Cladosporium sphaerospermum</i>	0/0	43/0	+	3	1	3	7	II
<i>Eurotium amstelodami</i>	4/0	0/0	-	1	3	1	5	I
<i>Fusarium oxysporum</i>	0/0	29/0	-	2	3	3	8	II
<i>Humicola</i> sp.	12/0	0/0	-	1	3	1	5	I
<i>Mucor plumbeus</i>	22/0	0/0	-	2	3	3	8	II
<i>Paecilomyces lilacinus</i>	0/0	5/0	-	1	3	3	7	II
<i>Paecilomyces variotii</i>	35/0	10/0	-	2	3	4	9	II
<i>Penicillium atramentosum</i> *	92/0	20/33 ^l	-	5	6	3	14	III*
<i>Penicillium brevicompactum</i>	0/0	0/33 ^l	+	2	2	3	7	II
<i>Penicillium implicatum</i>	15/0	0/0	-	1	3	3	7	II
<i>Penicillium canescens</i>	0/0	0/50 ^l	-	3	4	3	10	II
<i>Penicillium chrysogenum</i>	0/0	64/0	+	4	1	3	8	II
<i>Penicillium commune</i>	0/0	2/0	+	1	1	3	5	I
<i>Penicillium echinulatum</i>	15/0	0/0	-	1	3	3	7	I
<i>Penicillium frei</i> *	19/10 ^l	86/0	+	5	5	3	13	III*
<i>Penicillium glabrum</i>	0/0	2/0	-	1	3	3	7	II
<i>Penicillium griseofulvum</i>	73/0	0/0	+	4	1	3	8	II
<i>Penicillium hirsutum</i> *	54/0	0/50 ^l	-	3	6	3 ^b	12	III*
<i>Penicillium lividum</i> *	88/0	0/0	-	5	3	3	11	III*
<i>Penicillium oxalicum</i>	0/0	36/0	+	2	1	3	6	I
<i>Penicillium palitans</i>	33/0	0/0	-	2	3	3	8	II
<i>Penicillium polonicum</i>	0/0	46/0	+	3	1	3	7	II

Table 6. Classification of microorganisms from the work environment in tanneries into groups of indicators of microbial contamination – cont.
Tabela 6. Mikroorganizmy środowiska pracy w garbarniach vs grupy wskaźników zanieczyszczenia mikrobiologicznego – cd.

Microorganism Mikroorganizm	Frequency of occurrence in all of the samples in tanneries (air/surfaces) Częstość występowania we wszystkich próbach w garbarniach (powietrze/powierzchnie) [%]		Presence in the outdoor air Obecność w powietrzu zewnątrznym	isolation frequency częstość izolacji	Indicators of microbial contamination in tanneries Wskaźniki zanieczyszczenia mikrobiologicznego w garbarniach			group of indicators grupa wskaźników
	A	B			source źródło	harmfulness to human szkodliwość dla ludzi	total ogółem	
<i>Penicillium solitum</i>	17/0	0/0	+	1	1	3	5	I
<i>Penicillium verrucosum</i>[#]	0/40 ^l	86/100 ^l	-	5	6	3	14	III*
<i>Penicillium verruculosum</i>	0/0	0/50 ^l	-	3	4	3	10	II
<i>Rhizopus nigricans</i>	19/0	5/0	+	1	1	3	5	I
<i>Sclerocleista ornata</i>	0/0	0/33 ^h	-	2	4	1	7	II
<i>Stemphylium</i> sp.	0/0	0/50 ^l	-	3	4	1	8	II
<i>Trichoderma koningi</i>	0/0	17/50 ^l	+	3	5	1	9	II
<i>Trichoderma viride</i>	19/0	0/0	-	1	3	3	7	II
Yeast / Drożdże								
<i>Candida parapsilosis</i>	62/30 ^l	0/0	-	4	6	3	13	III*
<i>Cryptococcus albidus</i>	78/100 ^l	0/0	-	5	6	3	12	III*
<i>Cryptococcus neoformans</i>[#]	72/100 ^l	0/0	-	5	3	3	11	III*
<i>Cryptococcus terreus</i>	83/0	0/0	-	5	3	1	9	II
<i>Kloeckera</i> spp.	0/0	30/67 ^l	-	4	6	1	11	III
<i>Kodamaea ohmeri</i>	27/100 ^l	0/0	-	5	6	1	12	III
<i>Pichia angusta</i>	0/100 ^l	0/0	-	5	4	1	10	II
<i>Rhodotorula glutinis</i>	17/0	0/0	+	1	1	3	5	I
<i>Rhodotorula minuta</i>	0/0	10/0	+	1	1	1	3	I

A – tanneries processing raw hides / garbarnie przetwarzające skóry surowe; B – tanneries producing chrome-tanned leathers / garbarnie przetwarzające skóry chromowo garbowane.
 l – leather / skóry garbowane; h – hides / skóry surowe.

* See Table 7 / Patrz tabela 7.

[#] Non-hemolytic enterotoxin (NHE) and hemolysin BL (HBL) were detected in a laboratory test / Niehemolityczna enterotoksyna (NHE) i hemolizyna BL (HBL) zostały wykryte w badaniach laboratoryjnych.

^h Ochratoxin A and other mycotoxin were detected in a laboratory test / Ochratoksyna A i inne mykotoksyny zostały wykryte w badaniach laboratoryjnych.

Indicators of microbial contamination at workstations in tanneries are bolded / Wskaźniki zanieczyszczenia mikrobiologicznego w garbarniach zostały pogrubione.

Table 7. Genetic analysis of indicators of harmful biological agents contamination in the examined tanneries
Tabela 7. Analiza genetyczna wskaźników zanieczyszczenia szkodliwymi czynnikami biologicznymi w badanych garbarniach

Microorganisms Mikroorganizmy	Identification by conventional methods* Identyfikacja konwencjonalnymi metodami*	Identification by genetic methods Identyfikacja metodami genetycznymi	Similarity of nucleotide sequences to the compared strains Podobieństwo sekwencji nukleotydowych do porównywanych szczepów	
			strain szczep	%
Bacteria / Bakterie	<i>Bacillus pumilus</i>	<i>Bacillus pumilus</i>	<i>B. pumilus</i> ATCC 7061	99
	<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i>	<i>B. subtilis</i> NBRC 104443	99
	<i>Bacillus cereus</i>	<i>Bacillus cereus</i>	<i>B. cereus</i> CMCC 63305	99
	<i>Corynebacterium accolens</i>	<i>Corynebacterium lubricantis</i>	<i>C. lubricantis</i> KSS-3Se	100
Fungi / Pleśnie	<i>Cladosporium cladosporioides</i>	<i>Cladosporium cladosporioides</i>	<i>C. cladosporioides</i> ATCC 11275	100
	<i>Penicillium atramentosum</i>	<i>Penicillium echinulatum</i>	<i>P. echinulatum</i> FRR 1151	99
	<i>Penicillium freii</i>	<i>Penicillium commune</i>	<i>P. commune</i> ATCC 10428	100
	<i>Penicillium hirsutum</i>	<i>Penicillium chrysogenum</i>	<i>P. chrysogenum</i> ATCC10106	99
	<i>Penicillium lividum</i>	<i>Penicillium commune</i>	<i>P. commune</i> CBS 311.48	100
	<i>Penicillium verrucosum</i>	<i>Penicillium crustosum</i>	<i>P. crustosum</i> ATCC 90174	99
	Yeast / Drożdże	<i>Candida parapsilosis</i>	<i>Candida parapsilosis</i>	<i>C. parapsilosis</i> ATCC 22019
<i>Cryptococcus albidus</i>		<i>Cryptococcus albidus</i>	<i>C. albidus</i> CBS 969	99
<i>Cryptococcus neoformans</i>		<i>Rhodotorula glutinis</i>	<i>R. glutinis</i> LEMI 150	99

* Conventional identification of filamentous fungi was carried out on the basis of macro- and microscopic observation based on taxonomic keys; bacteria and yeast identification was carried out with Analytical Profile Index (API) tests / Identyfikacja konwencjonalna pleśni prowadzona była na podstawie obserwacji makro- i mikroskopowych w oparciu o klucze taksonomiczne; identyfikację bakterii i drożdży przeprowadzono z użyciem testów bazujących na Indeksie Profilu Analitycznego (API).

bioaerosol particle size distribution in the air of Tannery I demonstrated that its working environment was dominated by small particle sizes of 1.1–2.1 μm . Both bacterial and fungal bioaerosol particles were the majority of all (24–37%).

In the case of bacterial particles, a fraction of an aerodynamic diameter of 2.1–3.3 μm (17–23%) was also significant. The smallest share in the air of up to 10% was observed for bacterial particle size of 0.65–1.1 μm (Figure 1). Percentages of individual bacterial bioaero-

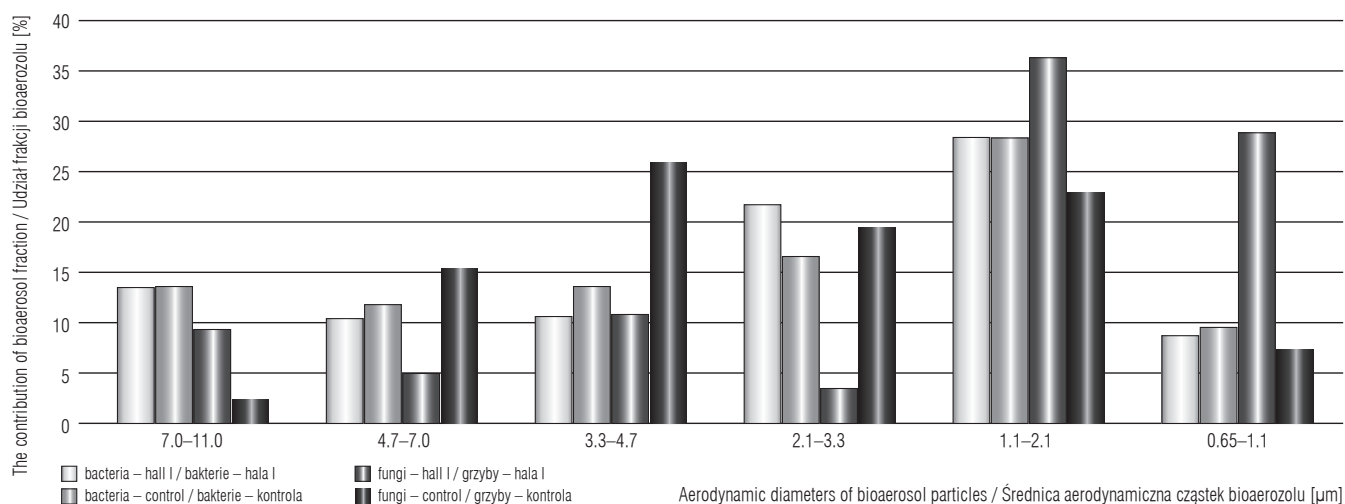


Fig. 1. The contribution of bioaerosol fraction of bioaerosol at workstations in tannery I
Ryc. 1. Rozkład frakcji bioaerozolu w bioaerozolu stanowisk pracy w garbarni I

sol fractions were similar in the production room and in the control sample (outdoor air). The fungal bioaerosol particle distribution was different for the indoor air of the tanneries and in the atmospheric air. Control samples were dominated by fractions of particles with sizes ranging from 1.1 to 4.7 μm . The smallest particles with diameters of 0.65–1.1 μm and 1.1–2.1 μm were dominant in the production air, constituting 24–37% of the total fungal bioaerosol. Also, a higher percentage of large particles (7.0–11.0 μm) in the production room was observed with respect to the control group.

DISCUSSION

Microclimate conditions prevailing in the working environment of tanneries (low temperature: 13–26°C and high humidity of the air: 40–90%) are conducive to the growth of microorganisms.

The microbial contamination of the air in tanneries, depending on humidity and temperature of the air in both types of tanneries (producing hides and chrome-tanned leathers) were described with linear regression equations (Table 8). The regression analysis of the correlation between humidity and temperature in the tanneries and microbial contamination of air gave the determination coefficients in the range of 0.6013 to 0.9193, indicating the significant influence of air humidity and temperature on the total number of microbes in the analysed air samples. The designated Pearson correlation coefficient for the examined correlations showed a positive value in the range of 0.78 to 0.96, therefore, it can be concluded that with the increase of humidity

and the temperature of the air the total number of microbes increases.

These studies demonstrated that microbial contamination of the air in the tanneries varied and depended on the characteristics of the plant and the used raw materials. The tanneries which processed raw hides and conducted a complete technological process involving advanced stages were characterized by about tenfold higher bacterial air contamination ($p < 0.05$), while the microbial contamination of leather surfaces was 10 000 times higher there than in the wet blue tanneries ($p < 0.05$).

One-way analysis of variance with *post-hoc* Tukey's test confirmed the significant influence of tanneries profile production on microbiological contamination of the surfaces of hides and leathers as well as the air samples (expressed as the total number of microorganisms).

The maximum levels of air pollution reported in the tanneries implementing a full technological process (bacteria: 4.0×10^3 – 1.1×10^4 CFU/m³, fungi: 1.7×10^3 – 2.8×10^4 CFU/m³) indicate high inhalation exposure to biological agents. This confirms the status of the control air (outdoor air) which, due to the number of microorganisms, in accordance with PN-89/Z-04111/02 and PN-89/Z-04111/03 (28,29), was clean air (less than 1.0×10^3 CFU/m³ of bacteria and less than 3.0×10^3 CFU/m³ of fungi) (Table 4) ($p < 0.05$). These results clearly show that the level of pollution in plant premises is influenced by the used technological process. However, the number of microorganisms in the air of the test plants did not exceed the quantitative

Table 8. Regression coefficients defining the correlation between microbiological contamination of air in the examined tanneries and microclimate conditions (humidity and temperature)

Tabela 8. Współczynniki regresji opisujące zależność między liczbą drobnoustrojów w powietrzu w badanych garbarniach a warunkami mikroklimatu (wilgotność i temperatura)

Parameter Parametr	Linear parameters of regression equations Współczynniki liniowe równań regresji ($y = ax+b$)		R ²	r	p
	a	b			
	Humidity				
tanneries processing raw hides / garbarnie przetwarzające skóry surowe	154.2	-3 484.9	0.9	0.9	< 0.05
tanneries producing chrome-tanned leathers / garbarnie przetwarzające skóry chromowo garbowane	1 153.0	-45 272.2	0.6	0.9	< 0.05
Temperature					
tanneries processing raw hides / garbarnie przetwarzające skóry surowe	551.0	-6 825.0	0.9	0.9	< 0.05
tanneries producing chrome-tanned leathers / garbarnie przetwarzające skóry chromowo garbowane	3 237.6	-53 620.3	0.7	0.7	< 0.05

R² – determination coefficient / współczynnik determinacji; r – correlation coefficient / współczynnik korelacji.

reference values established by the Polish Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment (30) (for the total number of mesophilic bacteria of 1.0×10^5 CFU/m³, and the total number of fungi 5.0×10^4 CFU/m³).

The percentage of microorganisms in the air was different, depending on the type of the plant. In Tanneries III and IV, processing raw hides, bacteria frequencies ranged from 63 to 92%, compared to the other test plants: 41–59%. This fact is most likely the result of processing material (raw hides) being susceptible to bacterial attack and high humidity caused by the processes conducted in tanneries. The analysis of bacteria on the leather surface showed a significantly higher contamination of raw hides than wet blue leathers. Raw hides, during the study, were only contaminated with bacteria.

The results of the bioaerosol particle size distribution measurement in the tannery air are especially noteworthy. The bacterial particle size distribution in the indoor air was similar to that in the outdoor air. In the case of fungi, there were significant differences ($p < 0.05$) between the production and control air. It was found that the smallest (fractions: 0.65–1.1 μm and 1.1–2.1 μm) and the largest test particles (7.0–11.0 μm) were found dominant in the production hall. This phenomenon is likely to arise due to the grouping of fungi in units of dust created during production (large particles) or the lifting of single mould spores with the movement of air (small particles).

The analysis of fungal bioaerosol distribution in the tannery indicates that the dominant fractions of aerodynamic diameters of 0.65–1.1 μm and 1.1–2.1 μm (together accounting for 67%) may penetrate the pulmonary alveolus of tanners, which may consequently lead to the occurrence of unspecific airways inflammation and allergic disorders (31). Moreover the exposure to the mould's β -D-glucan may cause inflammation reactions in lymphocytes, affects interleukin-1 secretion via T-lymphocytes, stimulates tumour defence mechanisms, causes a decrease in the numbers of pulmonary macrophages and inhibits phagocytosis (32). Consequently, the effects of glucan can lead to fatigue, headaches and other neurological symptoms in exposed workers, especially in the municipal waste industry and in different branches of agriculture (33,34).

Twelve species of microorganisms were selected as indicators for the working environment of the tanneries. They indicated a potential health hazard to workers and were associated with the specificity of raw mate-

rial and the working conditions in the tanneries. They included 4 bacteria: *B. pumilus*, *B. subtilis*, *B. cereus*, *Corynebacterium lubricantis*, 5 moulds: *C. cladosporioides*, *Penicillium commune*, *P. echinulatum*, *P. chrysogenum*, *P. crustosum* and 2 species of yeasts: *Candida parapsilosis* and *Cryptococcus albidus*. Genetic identification of yeasts initially classified as *Cryptococcus neoformans* showed that they belonged to the species of *Rhodotorula glutinis*.

No data is available on the adverse effects of this yeast to human health, which is why it is not presented as an indicator species. The genetic analysis also indicated differences in the taxonomic classification of 1 bacteria species from genus *Corynebacterium* and even 5 mould species belonging to the genus *Penicillium* selected as indicators of microbial contamination in the tanneries. The usefulness of molecular biology methods in the diagnostics of moulds isolated from the air has been proven (35,36).

Microorganisms designated as indicators of contamination with harmful biological agents in the tanneries are described in the literature as potentially pathogenic and dangerous to human health. The indicators included up to 3 species of the genus *Bacillus* (*B. subtilis*, *B. cereus*, *B. pumilus*). The presence of these bacteria had been previously identified as typical for leather (4) and air microflora in tanneries (37). *B. subtilis* has allergenic effects particularly burdensome for the employees of the biotechnology industry (production of enzymatic proteins) (38). This species is classified by the Regulation of the Minister of Health, 2005 (Journal of Laws of 2005, No. 81, item 716, as amended and the Journal of Laws 2008, No. 48, item 288) as a risk group 2 (17).

Another bacterium of this type, *B. cereus*, may exhibit toxic effects (it produces enterotoxins causing food poisoning), and infectious diseases – pneumonia, in exposed tanners through contact with food, airborne dust and airborne droplets (39). The ability to induce α -haemolysis and toxicity was detected for the strain isolated from the tanneries – laboratory tests showed a non-hemolytic enterotoxin (NHE) and hemolysin BL (HBL) (unpublished data). *Bacillus pumilus* is also considered an etiologic agent of food poisoning, while in the case of direct contact, it can cause skin infections (40).

Corynebacterium lubricantis bacteria are responsible for opportunistic infections in humans – Directive 2000/54/EC classifies all of the species of this genus as risk group 2 (16).

Designated as an indicator of microbial contamination in the tanneries, *C. cladosporioides* is a common

cause of allergic diseases in people exposed to the inhalation of bioaerosol in which they occur. *C. cladosporioides* allergens have been characterized in terms of structure and biological function by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS), the Allergen Nomenclature Sub-committee (41).

Dutkiewicz et al. (2007) (13) indicate that occupational hazard caused by the moulds of the genus *Penicillium* may lead to alveolitis allergica, bronchial asthma, allergic rhinitis and discomfort caused by toxicity in exposed workers. These moulds are known producers of citrinin, citreoviridin, cyclopiazonic acid, secalonic acid D, patulin, rubratoxin A and B, viridiatin (21). Laboratory tests showed that *Penicillium chrysogenum* moulds designated as the indicators of contamination with harmful biological agents in the tanneries produce a number of toxins, including ochratoxin A, aurofusarin, brevianamid F, brevicompanine B, chrysophanol, emodin, melegarin, roquefortine C or secalonic acid D (author's unpublished data).

The yeasts recognized as indicators of contamination with harmful biological agents in the tanneries included the species of *C. albidus* and *C. parapsilosis*. *C. albidus* may show invasive activity, it was isolated from the samples of human material, taken especially from hospitalized patients belonging to the so-called high risk groups (reduced immunity), *C. parapsilosis* has the ability to cause opportunistic fungal infections. These are usually the diseases of the mucous membranes, skin, internal organs and systemic diseases and sepsis (42).

The analysis of the air and leather surfaces in the tanneries has shown that the designated indicators of contamination with harmful biological agents are associated with the specific working environment. Their primary source, as indicated by the literature, may be animal material, soil and dust. The studies showed that the prevalence of the indicator isolates in the production space was high and reached 19–100% in the air and 10–100% on leathers, depending on the isolates.

CONCLUSION

The assessment of microbial contamination in the working environment of the tanneries showed increased numbers of bacteria and fungi in the air of production spaces in relation to the outdoor air. The level of microbial contamination is influenced by a technological process and the processed material. The studies

conducted in the 2 types of tanneries demonstrated that those processing raw hides had a higher contamination of the air and leather surface (1 and 4 orders of magnitude on the logarithmic scale) compared to the wet blue leather manufacturers. Bacteria were dominant among the microorganisms isolated in the tanneries. The bioaerosol particle size distribution in the production space of the tanneries indicates health risks arising from the possibility of inhaling fungal particles of sizes 0.65–1.1 µm and 1.1–2.1 µm, which may adversely affect the respiratory system of workers.

Basing on the prevalence, the source of isolation and health risk to people, 11 indicator microbial species were determined for the tanneries: *B. pumilus*, *B. subtilis*, *B. cereus*, *C. lubricantis*, *C. cladosporioides*, *P. commune*, *P. echinulatum*, *P. chrysogenum*, *P. crustosum*, *C. parapsilosis*, *C. albidus*. It has been shown that these are the species described in the literature as potentially pathogenic and associated with the specific technological processes in tanneries.

Although there were no exceedances of the permitted number of microorganisms recommended by the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment, the presence of microorganisms that could compromise the health of workers in the working environment was observed. Personal protection equipment should be used in tanneries, for instance, FFP3 half-masks that will protect workers from inhaling organic dust, chemicals and bioaerosol. Bioactive half-masks showing bacteriostatic and biocidal effects on microorganisms may also be suitable. It is recommended to develop guidelines for the control of harmful biological agents and methods for removing biological threats for this type of plants.

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