

DETERMINATION OF FUNGI RESISTANCE OF SEVERAL SAMPLES
OF NATURAL LEATHER IN CONDITIONS OF ARMENIA

I. V. SHAHAZIZYAN, I. M. ELOYAN, R. E. MATEVOSYAN *, S. G. NANAGULYAN

Chair of Botany and Mycology, YSU, Armenia

In this work we studied the effect of microscopic soil fungi on some leather samples. The species composition of micromycetes inhabiting the samples under study was revealed, and an assessment of the fungal resistance of the materials under study was given. In the process of work, species of microscopic soil fungi were isolated and identified from the soil. In order to determine the degree of resistance to fungi for the infection of samples, a water-spore suspension was obtained. The leather materials were partially destroyed by microscopic fungi, and mold resistance ranged from 2 to 3 on a 5-point scale.

<https://doi.org/10.46991/PYSU:B/2021.55.2.172>

Keywords: micromycetes, soil fungi, leather, biodeterioration, biodegradation, fungal resistance.

Introduction. Biodeterioration is any change (violation) of the structural and functional characteristics of an object caused by a biological factor. The destruction of polymeric materials under the influence of metabolic products of microorganisms occurs as a result of various reactions of oxidation, recovery, decarboxylation, etherification, hydrolysis, etc. [1].

The problem of biodeterioration is comprehensive in scientific meaning and diversified in practical terms. It includes both the study of the mechanisms of biodeterioration, the choice of measures to protect materials from biodestruction, and the development and application of methods for the study of biological damage. The variety of methods is associated, on the one hand, with a wide range of organisms that are agents of biodeterioration, and, on the other hand, with a wide spectrum of test materials [2]. The development and vital functions of microorganisms are closely related to the conditions of the environment in which they live. The external environment can stimulate or suppress the growth of biodestructors. The destruction of materials by fungi depends on their composition, primarily materials containing nutrients for fungi are damaged [3]. The mycelial structure of fungi is one of the most important biological features that determine the specific nature of their relationship with the environment. The mycelium quickly spreads over the substrate and covers large areas [4].

* E-mail: matevosyanruzanna@ysu.am (corresponding author)

The problem of biodeterioration of natural materials, especially leather for the upper of shoes, used in high humidity conditions, is of great importance. Microscopic fungi contribute to the increase of hygroscopicity of the leather, thereby increasing the relative humidity inside the shoe. This contributes to the early wear of the seams, as well as the development of pathogens inside the shoes. It should be noted that biodeterioration of polymer materials is closely related to the problem of human ecology, since many active biodegradants fungi are opportunistic organisms that can cause serious human diseases [5].

The aim of presented work was to study the biostability of leather materials in relation to microscopic fungi. The objectives of the study included identification of micromycetes isolated from various leather samples, determination of the fungal resistance of materials to the effects of mold fungi, isolated from various soils, identification of the most aggressive agents of biodeterioration of leather samples, and study of the fungicidal impact of materials.

Material and Methods. Various samples of natural leather were used as objects of research. To isolate microscopic fungi, a technogenically contaminated soil was used, a feature of which is a change in the number and species diversity of fungi. Research was carried out on the species composition of micromycetes isolated from soils contaminated with heavy metals near the town of Kajaran (Republic of Armenia), where the copper-molybdenum plant is located. Soil samples taken in the zone of technogenic pollution were studied. Soil samples were taken from a layer of 0–10 *cm*. The identification of microscopic fungi was carried out on the basis of cultural and morphological characters using generally accepted determinants. The specific names were specified according to the updated lists of species in the “Index Fungorum” database (<https://www.indexfungorum.org>). For mycological isolation of micromycetes and their analysis, the serial dilution method was used, which is based on the use of water-soil suspension and its transposition on the agar medium [6].

For determining micromycetes we used binocular magnifying MBS-9, and digital microscope ML-300 VWR. Some fungi images were made by digital microscope and computer software. Identification of soil microscopic fungi was done by using different determinants [7–11].

Tests for fungal resistance of samples to the action of mold fungi were carried out in accordance with GOST 9.049-91 “Unified system of corrosion and ageing protection. Polymeric materials and their components. Methods of laboratory tests for mould resistance (Method 1)” [12]. This method makes it possible to assess the fungal resistance of materials, i.e., the possibility of their use by micromycetes as food sources. Leather samples were placed in Petri dishes and inoculated with a suspension of fungal spores, placed in a thermostat, during 72 days, at $28 \pm 2^\circ\text{C}$ and humidity $> 95\%$. Fungal resistance was assessed in points based on a visual assessment of the growth rate of fungi on the test samples. At the same time, the assessment of the growth of fungi on leather samples was carried out on a five-point scale in accordance with the characteristics of points adopted by GOST 9.048-89 “Unified system of corrosion and ageing protection. Technical items. Methods of laboratory tests for mould resistance” [13]. 5 points are assigned to a sample with

visually determined development of fungi on an area of more than 25% of the surface, and 0 points - in the absence of fungal development, visible under a microscope (Tab. 1). A material is considered fungal resistant if it scores 0–2 points according to Method 1.

Table 1

Score	Characteristics of score
0	no spores and conidia germination were found under the microscope
1	germinated spores and slightly developed mycelium are visible under the microscope
2	developed mycelium are visible under the microscope, sporulation is possible
3	with the naked eye, mycelium and/or sporulation are barely visible, but clearly visible under a microscope
4	the development of fungi covering less than 25% of the test surface is clearly visible to the naked eye
5	with the naked eye, the development of fungi is clearly visible, covering more than 25% of the test surface.

The following species of fungi were used as test cultures: *Aspergillus niger*, *Penicillium cyclopium*, *Fusarium culmorum*, *Alternaria alternata*, *Stemphylium botryosum*, *Rhizopus stolonifer*, *Mucor sp.*

The concentration was checked using Goryaev's camera. Fragments of leather samples 5×5×5 cm in size (in 3 replicates) were cleaned from external contaminants. The samples were infected with a mixed suspension of 7 species of molds. After infection, the samples were placed in a thermostat, where a constant air humidity of 90% was maintained and kept for 72 days at 28°C.

The test samples were placed on a nutrient medium (agar medium) in Petri dish, which made it possible to reveal the fungistatic or fungicidal activity of the samples, as well as directly into sterile Petri dish in order to determine whether the tested leather samples are a food source for fungi. Mycological examinations of samples contaminated with a water-spore suspension with a set of test cultures of fungi were carried out every 5 days.

Results and Discussion. In total, 18 species of microscopic fungi were isolated from soil samples, which belong to 5 families, 4 orders, 4 classes and 2 divisions. In the soils were dominated by species of the genera *Aspergillus*, *Penicillium*, *Fusarium* (Tab. 2).

The determination of polymeric materials for resistance to micromycetes was carried out using the methods of GOST 9.049-91, according to which the samples of the studied materials were infected with suspensions of fungal spores. They were isolated from soils contaminated with heavy metals near the town of Kajaran. Leather samples were tested in two versions – with medium and without culture medium.

Table 2

Taxonomical composition of detected soil micromycetes

Division	Subdivision	Class	Order	Family	Genus	Species
Zygomycota	Mucoromycotina	Mucoromycetes	Mucorales	Mucoraceae	<i>Mucor</i>	<i>M. genevensis</i>
				Rhizopodaceae	<i>Rhizopus</i>	<i>Rh. microsporus</i>
						<i>Rh. stolonifer</i>
Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Aspergillaceae	<i>Aspergillus</i>	<i>A. clavatus</i>
						<i>A. flavipes</i>
						<i>A. flavus</i>
						<i>A. niger</i>
						<i>A. ochraceus</i>
						<i>A. versicolor</i>
						<i>A. wentii</i>
						<i>P. canesceas</i>
		Sordariomycetes	Hypocreales	Nectriaceae	<i>Fusarium</i>	<i>F. avenaceum</i>
						<i>F. oxysporum</i>
						<i>F. solani</i>
						<i>S. ilicisi</i>
Dothideomycetes	Pleosporales	Pleosporaceae	<i>Alternaria</i>	<i>A. alternata</i>		
				<i>Stemphylium</i>		

As seen in Fig. 1 in Petri dishes with a nutrient medium on the 7th day of research, a significant growth of fungi was observed.

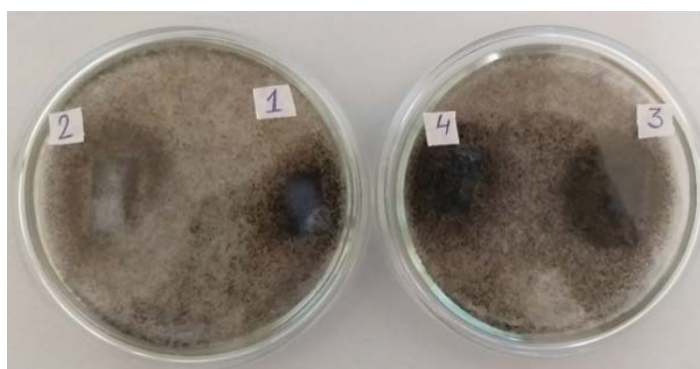


Fig. 1. Growth of micromycetes in Petri dishes with a nutrient medium.

However, on the 7th day of the study, no fungal growth was observed in Petri dishes without nutrient medium (Fig. 2).

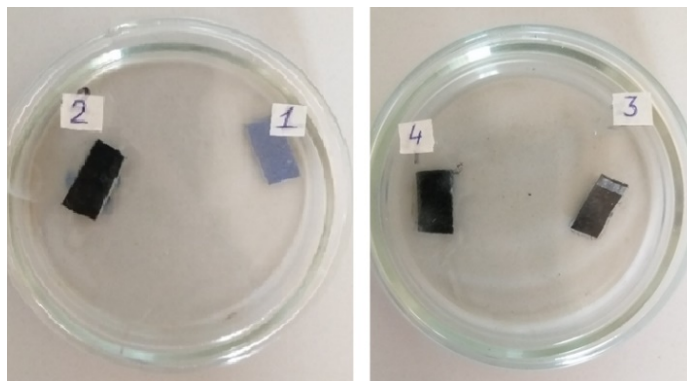


Fig. 2. Growth of micromycetes in Petri dishes without nutrient medium 1-4 samples of leather.

On the 30th day of the experiment, the growth of microscopic fungi continued in Petri dish with a nutrient medium and without medium (Fig. 3).

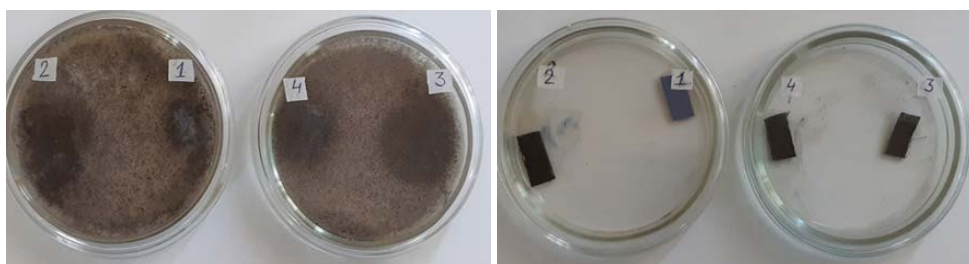


Fig. 3. 30th day of test.

Visual examination of the samples after 42 days of incubation showed signs of fungal growth on the examined samples of natural leather. As a result of exposure to microscopic soil fungi in Petri dishes without a nutrient medium, a change was found in the facial surface of the leather, which was damaged after a certain period of time. In case of bioprocessing, the front surface of the leather samples was overgrown with a cover of white and green, the shine disappeared and a gray pigmentation of the substrate was found. We have determined the composition of microorganisms that form well-visible plaques on the materials under study.

Fungi of the genera *Aspergillus*, *Penicillium*, *Trichoderma*, etc. have shown the greatest activity in affecting the studied leather samples. This fact can be explained by the fact that most species of the genera *Penicillium* and *Aspergillus* belong to the group of fungi that produce organic acids into the environment – the strongest aggressive metabolites of micromycetes that cause destruction various materials [14, 15].

As a result of the studies carried out during a visual examination of the samples on the 65th day, it was established that plaques of olive and dark gray (almost black) color appeared on the leather samples (Fig. 4).

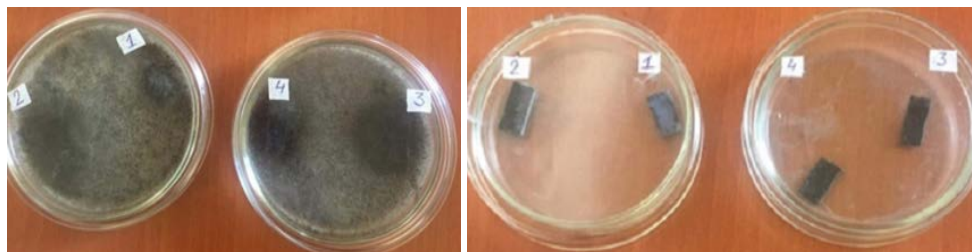


Fig. 4. 65th day of test 1–4 samples of leather.

On all leather samples, these plaques are formed by a complex of microscopic fungi *Aspergillus niger*, *A. ochraceus*, *A. clavatus*, *A. flavus*, *Rhizopus stolonifer*, *Rhizopus microsporus*, *Mucor genevensis*. As a result of mycological analysis, 7 species of fungi were isolated and identified. Zygomycetes were represented by 3 species, ascomycetes – 4 species (Tab. 3). The amount of isolated fungi increases with the development of the destructive process.

Table 3

Species composition of detected micromycetes by taxonomical groups

Division	Class	Order	Family	Genus	Species
Zygomycota	Mucoromycetes	Mucorales	Mucoraceae	<i>Mucor</i>	<i>M. genevensis</i>
			Rhizopodaceae	<i>Rhizopus</i>	<i>Rh. microsporus</i>
					<i>Rh. stolonifer</i>
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	<i>Aspergillus</i>	<i>A. clavatus</i>
					<i>A. flavus</i>
					<i>A. niger</i>
					<i>A. ochraceus</i>

These species of fungi are found in all Petri dishes; therefore, the studied samples contain nutrients that provide an insignificant development of fungi and have 2 and 3 points of damage.

The identified fungi have a large absorption surface and have an active effect on the environment through the products of metabolism.

On the 72nd day of the experiment, further growth of fungi are observed in Petri dishes with a nutrient medium, and in Petri dishes without a nutrient medium, the growth of fungi is activated, but the process of biodegradation is not observed (Fig. 5).

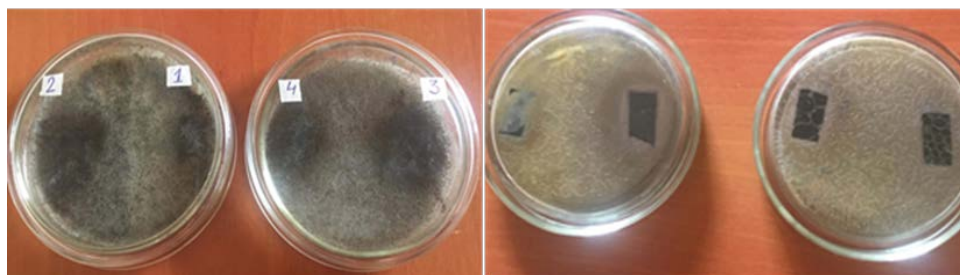


Fig. 5. 72nd day of test.

During our investigation, it was revealed that the properties of the leather tissue change when exposed to microorganisms. As follows from the data obtained, as a result of the action of microorganisms, the studied properties of the leather change noticeably, while microscopic fungi of the *Aspergillus niger* species have a stronger destructive effect on the leather.

It is known that many species of the genus *Aspergillus* have a high metabolic activity and an adaptive ability to use a wide variety of organic compounds, including those that are almost not metabolized by other organisms. Some authors indicated that 66 enzymatic reactions for the transformation of various compounds, carried out only by *A. niger* [10].

It is known that produced microorganisms and organic acids, enzymes, pigments and some other metabolites cause significant changes in the physico-mechanical and other properties of materials, and sharply worsen their technological parameters. It should be noted that leather products can pose a potential danger to consumers, since all isolated micromycetes are opportunistic.

Thus, in the case of object destruction in the environment, external conditions such as temperature, pH of the environment, existing relevant microorganisms, and their interaction with the surface of the destroyed object are genuinely essential.

As an outcome of studies carried over the fungal leather samples tenacity, several stages of material destruction were found – adhesive binding of microorganisms on the surface, its colonization, fouling, surface bioerosion, partial biodegradation of the object. It should be noted that biodegradation does not engage merely one species in the process but an association of Mucoromycetes. They mutually influence each other and produce enzymes involved in the material's destruction process.

An experiment to study the effect on microscopic fungi leather samples made it possible to identify qualitative destruction indicators. At the first stage of the destruction process, the material partially softens, which increases the possibility of microorganisms' functionality. Then, the process is followed by the viscosity of microscopic fungi on the sample surface, a partial penetration of the mycelium deep into the material, and intensive reproduction of microscopic fungi. The latter indicates that the content of microscopic fungi used in the study samples is a nutrition source.

The study results manifest the practicality of usage of biodegradation technology together with microscopic fungi aiming for partial utilization of leather waste. It unveils, the examined leather samples contain nutrients enabling microscopic soil fungi growth and development. It is also worth mentioning, majority of the identified molds belong to microorganisms of III and IV pathogenicity groups according to CP 1.3.2322-08. Therefore, leather products and waste-infested moldy fungi can pose a potential danger to humans, causing many fungal diseases.

Based on the studies carried out on the biodegradation of leather materials, the following conclusions can be drawn:

- the leather materials are subject to microscopic fungi partial destruction, while the tenacity of moldy fungi has varied from 2 to 3 points on a 5-point scale according to GOST 9.049-91;

- the main biodestructors of investigated materials are *Aspergillus* and *Penicillium* species;
- visual assessment of the destruction degree of the samples demonstrated a significant change in the mechanical properties of materials after 15 days of the experiment (softening, fiber release, delamination).

Received 16.06.2021

Reviewed 30.07.2021

Accepted 11.08.2021

REFERENCES

1. Sakhno O.N., Selivanov O.G., Chukhlanov V.Yu. *Biological Resistance of Polymeric Materials*. Vladimir, Vladimir. Gos. Univ. imeni A.G. i N.G. Stoletovikh (2014), 64 p. (in Russian).
2. Kryazhev D.V., Smirnov V.F., et al. Methods of Testing Industrial Materials for Bioresistance (An Analysis of Criteria and Approaches). *Vestnik of Lobachevsky University of Nizhni Novgorod* **2**(1) (2013), 118–124 (in Russian).
3. Yerofeyev V.T., Bogatov A.D., et al. Research of the Biological Firmness of Building Materials with Provision for Their Deterioration. *Vestnik VolgGASU. Ser. Stroit. Arhit.* **22**(41) (2011), 73–78 (in Russian)
4. Pekhtasheva E.V., Neverov A.N., et al. Biodegradation and biodeterioration of materials. Who is responsible for this? *Bulletin of the Kazan Technological University* **15** (2012), 222–233. (in Russian)
5. Antonov V.B. *Impact of Biodegradable Buildings on Human Health. Problems of Longevity of Buildings and Equipment in Modern Construction*. St. Peterburg (2007), 137–142. (in Russian)
6. Matevosyan R.E., Nanagulyan S.G., et al. Study of Soil Fungi in the Territory of Kapan City and Their Surrounding. *Proc. YSU B: Chem. Biol. Sci.* **52** (2018), 193–167.
<https://doi.org/10.46991/PYSU:B/2018.52.3.193>
7. Pidoplichko N.M. *Penicillium*. Kiev, Naukova Dumka (1972), 150 p. (in Russian)
8. Pidoplichko N.M., Milko A.A. *Atlas of Mucoral Fungi*. Kiev, Naukova Dumka (1971), 115 p. (in Russian).
9. Litvinov M.A. *Determinant of Soil Microscopic Fungi*. Leningrad, Nauka (1967), 303 p. (in Russian)
10. Bilai V.I., Koval E.Z. *Aspergillus*. Kiev, Naukova Dumka (1988), 390 p. (in Russian)
11. Koval E.Z., Rudenko A.V., Voloshuk N.M. *Penicillium*. Kiev, NNIRXV (2016), 407 p. (in Russian)
12. GOST 9.049-91. *Unified system of corrosion and ageing protection. Polymeric materials and their components. Methods of laboratory tests for mould resistance*. Committee of Standardization and Metrology of the USSR. Moscow (1991). (in Russian)
13. GOST 9.048-89. *Unified system of corrosion and ageing protection. Technical items. Methods of laboratory tests for mould resistance*. USSR State Committee for Standards. Moscow (1989). (in Russian)
14. Anisov A.A., Feldman M.S., Visockaya L.B. Mycelial Fungi Ferments as Aggressive Metabolites. In: *Biodeterioration in Industry*. Interuniversity Collection of Scientific Articles. Gorkiy, GSU (1985), 3–19 (in Russian)
15. Smirnov V.F., Semicheva A.C., et al. Aggressive Metabolites of Fungi and their Role in the Degradation of Materials in Various Chemical Compositions. In: *Materials of Conference on Biological Problems of Environmental Materials Science*. Penza (1995), 82–86. (in Russian)

Ի. Վ. ՇԱՀԱԶԻԶՅԱՆ, Ի. Մ. ԷԼՈՅԱՆ, Ռ. Է. ՄԱԹԵՎՈՍՅԱՆ, Ս. Գ. ՆԱՆԱԳՅՈՒԼՅԱՆ

**ՀԱՅԱՍՏԱՆԻ ՊԱՅՄԱՆՆԵՐՈՒՄ ԲՆԱԿԱՆ ԿԱՇՎԻ ՈՐՈՇ
ՆՍՈՒՇՆԵՐԻ ՍՆԿԱԴԻՄԱՑԿՈՒՆՈՒԹՅԱՆ ՈՐՈՇՈՒՄԸ**

Աշխատանքում ուսումնասիրվել է մանրադիտակային հողային սնկերի ազդեցությունը կաշվե մի քանի նմուշների վրա: Բացահայտվել է հետազոտվող նմուշների վրա բնակեցված հողային միկրոմիցետների տեսակային կազմը և գնահատվել է հետազոտվող կաշվե նմուշների սնկադիմացկունության աստիճանը: Մնկադիմացկունության աստիճանը գնահատելու համար հողից անջատվել և նույնականացվել են մանրադիտակային սնկերի տեսակներ: որոնցից պատրաստվել է ջրասպորային կախույթ և ցողվել փորձարկվող կաշվե նմուշները: Ըստ նյութերի սնկադիմացկունության գնահատման 5 միավորանոց սանդղակի փորձարկվող կաշվե նմուշների մոտ նկատվել է դիմացկունության տատանում 2–3 միավորի սահմաններում:

И. В. ШАХАЗИЗЯН, И. М. ЭЛОЯН, Р. Э. МАТЕВОСЯН, С. Г. НАНАГЮЛЯН

**ОПРЕДЕЛЕНИЕ ГРИБОСТОЙКОСТИ НЕКОТОРЫХ ОБРАЗЦОВ
НАТУРАЛЬНОЙ КОЖИ В УСЛОВИЯХ АРМЕНИИ**

В работе изучалось влияние микроскопических почвенных грибов на некоторые образцы кожи. Выявлен видовой состав микромицетов, населяющих исследуемые образцы, и дана оценка грибостойкости исследуемых материалов. В процессе работы из почвы были выделены и идентифицированы виды микроскопических почвенных грибов. Для определения степени устойчивости образцов к грибкам при заражении была получена водно-споровая суспензия. Кожаные материалы были частично разрушены микроскопическими грибами, а устойчивость к плесени варьировалась от 2 до 3 по 5-балльной шкале.