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Biology

ASSESSMENT OF THE BIODESTRUCTION OF SOME POLYMERIC COMPOSITE MATERIALS USED IN HOUSEHOLD

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In this research, we investigated the impact of micromycetes on polyethylene bags used for household waste disposal and packaging, plastic bottles, as well as bags designed for everyday use and food storage. As a result of mycological studies of soil samples 17 species of microscopic fungi belonging to 7 genera were isolated and identified: *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus*. It should be noted that the major part of species (13) belongs to the Ascomycota. Mucoromycota division is represented by 4 species. The taxonomic analysis of the mycobiota of the studied soil samples revealed that the most common genera in the samples were *Penicillium* and *Aspergillus*. This research provided the biodestruction potential of the studied polymer materials and has been observed that the duration of biodegradation can be influenced by changes in environmental conditions such as temperature, humidity and certain growth factors. Not all species of fungi growing on polymer materials are true destructors capable of using the material itself or its components as an energy source. The degradation of materials is not only attributed to a single specific species but rather to a complex interplay of various species of micromycetes.

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***Keywords*:** polymer, biodestruction, micromycetes, soil, fungal resistance.

Introduction. Polymeric materials have become an integral part of almost every aspects of human activity. Unfortunately, their extensive use has given rise to a significant environmental challenge, as polymers and their waste persist in the soil and the environment for extended periods, damage substantial harm. The urgency of addressing polymer disposal stems from the ever-increasing rates of plastic production and consumption, primarily in industrialized countries. This surge in polymer waste accumulation has led to a global problem [1].

As we continue to expand the application of polymer materials, especially those with short service lives like packaging for food, medicines, and consumer goods, the volume of polymer waste escalates dramatically, intensifying the issue of disposal [2]. An essential aspect of this challenge lies in effectively managing both industrial and household polymer waste. Therefore, a pressing scientific and technical imperative of our times is the development of new biodegradable polymeric composite materials for various industries, including agriculture and medicine.

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Notably, the creation of biodegradable polymer materials holds promise. These materials undergo physicochemical and biological transformations under the influence of environmental factors, and as a result the biodegradation products seamlessly reintegrate into the natural substance cycle [3–5].

In recent years, the chemical industry has witnessed remarkable growth, resulting in an annual production of 140 million tons of diverse polymers. Unfortunately, the majority of these polymers do not biodegrade, leading to their long-lasting presence in the environment [6].

Due to the significant accumulation of plastic bottles, bags, and various plastic wastes in landfills, there has been a pressing need to conduct a series of experiments aimed at evaluating the efficacy of biodegradation for commonly used packaging materials. In this context, the objective of this study is to assess the potential for the biological degradation of polymeric composite materials.

Materials and Methods. In our research, we focused on the investigation of various objects, including polyethylene bags used for household waste disposal and packaging, plastic bottles, as well as bags designed for everyday use and food storage.

We isolated microscopic fungi from polluted soil to create water-spore suspensions, which were essential for studying the resistance of different samples. Notably, the contaminated soil exhibited a high degree of fungal species diversity, a distinctive characteristic of technogenic waste pollution.

The evaluation of sample resistance to mold fungi was conducted in accordance with GOST 9.049-91, titled "Unified System of Corrosion and Aging Protection for Polymeric Materials and Their Components: Laboratory Testing Methods for Mold Resistance (Method 1)" [7]. This standard is applicable to a wide range of polymer-based materials, including compounds, rubbers, adhesives, sealants, as well as their constituent elements such as polymers, plasticizers, fillers, stabilizers, dyes, pigments, and more. It provides detailed descriptions of three distinct laboratory testing methods aimed at assessing resistance to mold fungi.

The objective of this method was to create and maintain optimal conditions for the growth of fungal spores on contaminated materials, subsequently allowing for the assessment of fungal resistance based on the extent of mold development.

The initial phase of our research involved the isolation of naturally occurring micromycetes, particularly from soil contaminated by technogenic waste. These fungi play an essential role in the natural degradation of polymer compositions. The identification of microscopic fungi was carried out based on cultural and morphological characters using generally accepted determinants [8–11].

The nomenclature was standardized in accordance with the latest species lists available on the "MycoBank" database (https://www.mycobank.org/). We used the serial dilution method to isolate micromycetes from the soil [12].

We prepared a suspension containing fungal spores with a concentration of 1–2 million /cm^3 per each species just prior to conducting the test. This suspension was made using distilled water.

According to the guidelines set forth in GOST 9.048-89 [13], though with certain modifications, we conducted an extensive 140-day experiment, as opposed to the standard 28-day duration. Sequentially, we applied drops of an aqueous spore suspension to the surface of the samples to induce fungal infection. The assessment of the samples' resistance to fungal degradation was performed using Petri dishes,

both with and without a nutrient medium. Following the inoculation of various fungal strains, the Petri dishes were transferred to a thermostat and maintained for 140 days at a controlled temperature of 28 ± 2°C and a humidity level of 90%. To monitor fungal growth, the samples were systematically examined at 7-day intervals throughout the 140-day testing period.

Results and Discussion. As a result of mycological studies of soil samples, 17 species of microscopic fungi belonging to 7 genera were isolated and identified: *Mucor, Rhizopus, Aspergillus, Penicillium, Fusarium, Alternaria, and Cladosporium*. It should be noted that the major part (13 species) belongs to the Ascomycota. Mucoromycota division is represented by 4 species (Tab. 1).

Table 1

Systematic analysis of mycobiota isolated from the studied soil samples

Division/ Subdivision	Class	Order	Family	Genus	Species
Mucoromycota/ Mucoromycotina	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*M.heterosporus* A. Fischer
					M. circinelloides Tiegh.
				Rhizopus	*Rh. stolonifer* (Ehrenberg) Vuillemin
					Rh. microsporus Tiegh.
Ascomycota/ Pezizomycotina	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus*	*A. niger* Tiegh.
					A. ochraceus K. Wilh.
					A. flavus Link
				Penicillium	*P. adametzii* K.W. Zaleski
					P. canescens Sopp
					P. corylophilum Dierckx
					P. cyclopium Westling
					P. lanosum Westling
					P. crustosum Thom
	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*F. chlamydosporum* Wollenw
					F. avenaceum (Fr.) Sacc.
	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*A. alternata* (Fr.) Keissl.
		Cladosporiales	Cladosporiaceae	*Cladosporium*	*C. herbarum* (Pers.) Link
2/2	4	5	5	7	17

According to certain authors, the isolation of 10 to 15 species of micromycetes from a single ecosystem is considered indicative of notably high fungal species diversity [14]. The vast majority of the isolated micromycetes species are typical saprotrophs from the Ascomycota division, which is introduced by 5 genera belonging to the orders *Eurotiales*, *Hypocreales*, and *Pleosporales*.

The taxonomic analysis of the mycobiota of the studied soil samples revealed that the most common genera in the samples were *Penicillium* and *Aspergillus*. The polluted soil mycobiota is characterized by the predominance in species diversity of representatives of the *Penicillium* genera – 6 species out of 17. The samples also contained species of the *Aspergillus* genus (3 species), which are typical inhabitants of soil in general. The *Fusarium* genus is represented by the species *F. avenaceum*, *F. sporotrichioides*. It should be noted that the most important factor in the abundance of *Fusarium* species in soil is the temperature. It is stated that the optimal temperature for the growth of *F. sporotrichioides* is 22–25°C, and for *F. avenaceum* growth is observed at 20°C.

The results gained by studying soil samples polluted by high levels of technogenic waste indicate the availability of dark-colored fungi. In particular, species of *Alternaria* and *Cladosporium* genera were noted, as well as representatives of Mucoromycetes (Fig. 1).

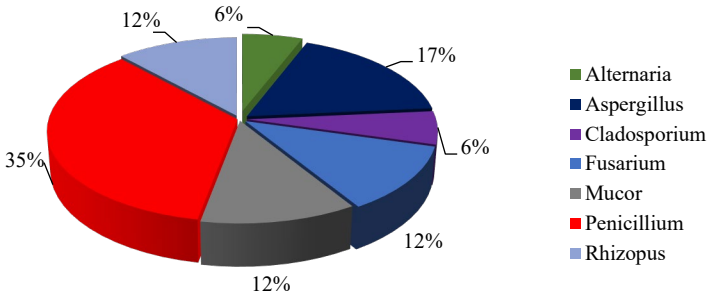

Fig. 1. Percentage of fungi separated from the soil.

The mycological analysis of soil samples contaminated with technogenic waste reveals the presence of potentially pathogenic fungal species. Notably, some of these identified species serve as primary agents in the biodegradation of diverse polymer materials.

To assess the biodegradation of the studied polymers, samples were prepared using the aforementioned method outlined in GOST 9-048-89.

We isolated test cultures from 6 species of microscopic fungi, which are known as key biodestructors, from the soil micromycetes: *Aspergillus niger*, *Alternaria alternata*, *Rhizopus stolonifer*, *Cladosporium herbarum*, *Fusarium sporotrichioides*, *Penicillium canescens*. After that, the water-spore suspensions from the pure cultures were made and the Petri dishes were artificially infected (Fig. 2).

Fig. 2. Petri dishes with micromycetes colonies.

Our research focuses on common packaging materials, specifically plastic bags and plastic bottles. We examined three samples: the first was a plastic bottle and the second and third were plastic bags.

Following the incubation period, we visually assessed the rate of biodegradation of composite materials resulting from mold fungi growth. The resistance of the samples to the collective action of fungi was evaluated in accordance with standard laboratory tests. The degree of growth of mold fungi on the surface of every sample was rated on a 5-point scale according to GOST 9.048-89 (Tab. 2).

Table 2

Point scale for assessing fungal resistance of materials

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

During the testing phase, we observed the initial growth of fungi, including mycelium formation, as early as the 3rd to 5th day within Petri dishes containing a nutrient medium. By the 7th day, the entire medium's surface was covered with mycelium, except for the areas where the polymer tape samples were situated (Fig. 3). It is noteworthy that during this testing method, the material was exposed to secondary metabolites released by the micromycetes during their growth on the nutrient medium, even if it did not serve as a direct nutrient substrate for the fungi.

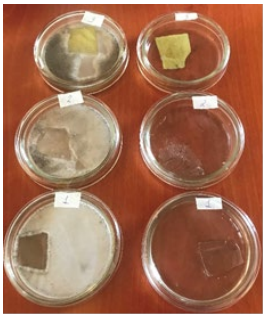

Fig. 3. 3th day of test

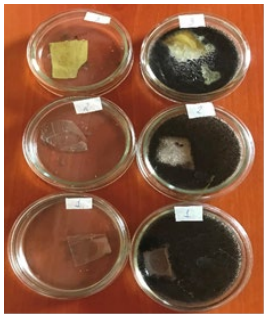

Fig. 4. 8th day of test

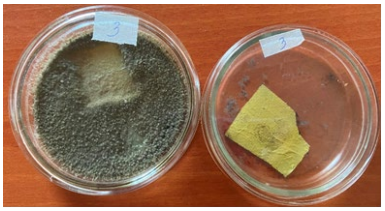

Fig. 5. 12th day of test.

In all three Petri dishes containing a nutrient medium, we detected the growth of fungi belonging to the order Mucorales. Notably, colonies of the species *Rhizopus stolonifer* were identified, and their exomycelium was visually observed. By the 8th day, in the Petri dishes without a nutrient medium, we observed slightly developed mycelium in colonies of *Mucor* sp. (Fig. 4).

Over the following four days, no significant changes were observed in samples 1 and 2, while sample 3 exhibited substantial fungal growth (Fig. 5).

In Petri dishes with a nutrient medium, the initial growth of mold fungi, including mycelium formation, was observed within the first week of incubation. Subsequently, there was active growth of microfungal colonies, yet the material itself was not entirely covered with mold fungi. It is worth noting that this fungal growth was sustained due to the presence of the nutrient medium, which serves as a substrate for the polymers. Consequently, the polymer materials were subjected to a more pronounced and sustained impact.

On the 28th day of the experiment, significant fungal growth was observed in all Petri dishes containing a nutrient medium. This occurrence can be attributed to the

partial removal of the aerial mycelium of *Rhyzopus* colonies, which, in turn, stimulated the active and intense growth of species from the *Mucor* and *Aspergillus* genera.

A notable transition of fungal colonies from the nutrient medium to the surface of the test polymer samples was also observed. This indicates that the most intensive formation of fungal biomass occurs at the boundary between the nutrient medium and the investigated polymer samples.

After 28 days of incubation in Petri dishes without a nutrient medium, we did not observe mycelial growth in any of the examined polymer samples. However, visible spore agglomerates were present, and the following fungal species were identified: *Aspergillus niger*, *Penicillium duclauxii*, and *Mucor* sp.

During the next 70 days, no change in the species composition of fungi occurred (Figs. 6–8). However, on the 95th day, we noted an increase in the growth of *Penicillium* genus representatives (Fig. 9). Notably, we identified a species *Penicillium duclauxii*, which had not been previously observed. Additionally, another *Penicillium* species was observed, although it remained unidentified. Notably, this particular fungus exhibited significant changes in its conidia. These changes may be of an adaptive nature, as it is well-documented that a polluted environment and extreme conditions can induce alterations in the structure of the conidial apparatus of fungi [15]. Thus, the main groups of microorganisms developing on the nutrient medium were microscopic fungi, the dominant genera of which were *Penicillium* and *Aspergillus*. Species of the genera *Mucor* and *Rhyzopus* were also identified.

Fig. 6. 30th day of test.

Fig. 7. 50th day of test.

Fig. 8. 60th day of test.

Fig. 9. 95th day of test.

Fig. 10. 140th day of test.

The identification of the isolated microscopic fungi revealed the presence of five predominant species: *Rhizopus stolonifer*, *Mucor* sp., *Aspergillus niger*, *Penicillium duclauxii*, *Penicillium* sp. These fungi were also detected on the studied samples in Petri dishes without a nutrient medium, indicating their role as biodestructors of the samples. They exhibited development on nearly all samples throughout the entire 140-day duration of the experiment (Fig. 10).

However, the species *Alternaria alternata*, *Fusarium sporotrichioides* and *Cladosporium herbarum* did not reveal any activity.

It's important to note that the damage inflicted on the samples by fungi is a gradual process. The results of the experiment highlight that the samples may contain nutrients that promote fungal growth. Biodegradation by fungi is indeed possible, but occurs over an extended period. Experiments on fungal resistance indicate that, in Petri dishes with a nutrient medium, the biodegradation process of the samples advances more rapidly, and the fungi exhibit a quicker colonization of the tested materials.

During the course of our research, we observed a transition in mold growth on the surface of the tested samples, characterized by visible circular patterns, indicative of sample degradation. Our observation suggests that these materials contain nutrients that can serve as a source of sustenance for the micromycetes. It's important to note that the degradation of materials is not only attributed to a single specific species but rather to a complex interplay of microorganisms. This phenomenon aligns with the findings of some researchers who propose that one group of microorganisms prepares a substrate for another through their activity [16].

Fungal enzymes and metabolites, in conjunction with water and soil components, contribute to the ongoing degradation of polymers. Consequently, the biodegradation process of the studied samples is protracted.

The growth of micromycetes was especially pronounced on these materials, and after standard tests, the intensity of fungal growth was visually assessed as 3 points (GOST 9.048-89). In Petri dishes without a nutrient medium, the presence of mycelium and sporulation is slightly visible to the naked eye but becomes more apparent under microscopic observation, which indicates the relative resistance of the substrate to fungal colonization.

Thus, our research provided valuable insights into the biodegradation potential of the studied polymer materials. It has been observed that the duration of biodegradation can be influenced by changes in environmental conditions such as temperature, humidity and certain growth factors.

The results of our research show that not all species of fungi growing on polymer materials are true destructors capable of using the material itself or its components as an energy source. Some strains may simply develop on these materials due to the presence of various organic contaminants on their surface.

Fungal activity was indeed observed in the samples, predominantly caused by species such as *Rhizopus stolonifer*, *Mucor* sp., *Aspergillus niger*, *Penicillium duclauxii, Penicillium* sp. However, the biodegradation process appeared to be gradual and dependent on the availability of nutrients in the materials. Notably, the use of a nutrient medium accelerated the biodegradation process, explains the complex interaction of various micromycetes that participate in material degradation.

Finally, this study highlights the importance of considering the relative fungal resistance of the substrate during assessing the biodegradation of polymeric

materials. This work enhances our understanding of the complicated dynamics of biodegradation and its potential for future exploration and practical applications.

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ԿԵՆՑԱՂՈՒՄ ՕԳՏԱԳՈՐԾՎՈՂ ՊՈԼԻՄԵՐԱՅԻՆ ԿՈՄՊՈԶԻՑԻՈՆ ՆՅՈՒԹԵՐԻ ԿԵՆՍԱՔԱՅՔԱՅՄԱՆ ԳՆԱՀԱՏՈՒՄ

Աշխատանքում ուսումնասիրվել է միկրոմիցետների ազդեցությունը պոլիէթիլենային տոպրակների, պլաստիկ շշերի, ինչպես նաև ամենօրյա օգտագործման և սննդի պահպանման համար նախատեսված պարկերի վրա: Ուսումնասիրությունների արդյունքում հայտնաբերվել և նույնականացվել է միկրոմիցետների 17 տեսակ, որոնք պատկանում են 7 ցեղերի՝ *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*: Հարկ է նշել, որ տեսակների զգալի մասը (13) պատկանում է պայուսակավոր սնկերին (բաժին Ascomycota): Մուկորայինների (Mucoromycota) բաժինը ներկայացված է 4 տեսակով: Հետազոտված հողի նմուշների միկրոմիցետների կարգաբանական վերլուծությունը ցույց է տվել, որ նմուշներում ամենատարածվածը *Penicillium* և *Aspergillus* ցեղերն են: Ուսումնասիրությունների արդյունքում բացահայտվել է ուսումնասիրված պոլիմերային նյութերի կենսաքայքայման ներուժը և պարզաբանվել, որ կենսաքայքայման գործընթացի տևողության վրա կարող են ազդել շրջակա միջավայրի պայմանների փոփոխությունները (ջերմաստիճանը, խոնավությունը և աճի որոշ գործոններ): Պոլիմերային նյութերի վրա աճող սնկերի ոչ բոլոր տեսակներն են հանդիսանում իսկական քայքայիչներ, որոնք կարող են օգտագործել նյութը կամ դրա բաղադրիչները որպես էներգիայի աղբյուր: Նյութերի քայքայումը կապված է ոչ միայն մեկ որոշակի տեսակի զարգացման հետ, այլ նաև պայմանավորված է միկրոմիցետների տարբեր տեսակների փոխազդեցությամբ:

Л. В. МАРГАРЯН, Р. Э. МАТЕВОСЯН, И. М. ЭЛОЯН,
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ОЦЕНКА БИОДЕСТРУКЦИИ НЕКОТОРЫХ ИСПОЛЬЗУЕМЫХ В БЫТУ ПОЛИМЕРНЫХ КОМПОЗИЦИОННЫХ МАТЕРИАЛОВ

В данном исследовании было показано влияние микромицетов на полиэтиленовые пакеты, используемые для утилизации и упаковки бытовых отходов, пластиковые бутылки, а также пакеты, предназначенные для повседневного использования и хранения продуктов питания. В результате микологических исследований образцов почвы выделено и идентифицировано 17 видов микроскопических грибов, принадлежащих к 7 родам: *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*. Следует отметить, что большая часть видов (13) принадлежит отделу Ascomycota. Отдел Mucoromycota представлен 4 видами. Таксономический анализ микобиоты изученных образцов почвы показал, что наиболее распространенными родами являются *Penicillium* и *Aspergillus*. Исследование выявило потенциал биодеструкции изученных полимерных материалов и показало, что на продолжительность биоразложения могут влиять изменения условий окружающей среды, таких как температура, влажность и определенные факторы роста. Не все виды грибов, растущие на полимерных материалах, являются настоящими деструкторами, способными использовать сам материал или его компоненты в качестве источника энергии. Деградация материалов связана не только с одним конкретным видом, но и со сложным комплексом взаимодействия различных видов микромицетов.