Features of Biological Effects on Polymer Composite Materials in the Climatic Conditions of Yakutia

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Abstract. A large variety of microorganisms was isolated. It has been established that the soil cover and atmospheric air in the area of polygon climatic tests in the city of Yakutsk contain various types of microorganisms, including pathogenic species of mold fungi of the genus Aspergillus, which are highly likely to have biological agents of experimental samples in contact with soil and atmospheric air in the landfill. What is confirmed by the results of microbiological studies.

1. Introduction
At present, innovative polymer composite materials (PCM) are multi-element substances that require a more comprehensive approach to the study of their physico-mechanical properties and resistance to biodeterioration, especially when used in cold climates. The specifications of aging and destruction of materials operated at extremely low temperatures are due to a number of climatic influences. Investigation of the stability of PCM components and finished products under the influence of abiogenic and biogenic factors under low-temperature conditions based on the multilevel impact of high-quality components, resulting in the formation of future products, mixed components, in the native state and after experimental exposure to complexes of agents that cause aging and materials, under the influence of artificially created and indigenous associations of microorganisms.

The purpose of this research was to study the features of biological effects on PCM in the climatic conditions of Yakutia.

2. Materials
The research material was fragments of bio-damaged and undamaged PCM, soil, snow cover, and atmospheric air samples taken in the area of the experimental samples.

Sampling was performed three times on September 11, 2018, December 12, 2019, January 14, 2020.

The study of the numerical and qualitative composition of microorganisms and their identification was carried out in accordance with generally accepted methods in microbiology, instructions and guidelines.
We used a laboratory polarized microscope Axiolab Pol, manufactured by «Karl Zeiss» Germany, an N-Achromat lens 5 × / 0.13 (color code - red, free working distance (FOD) - 11.2 mm). Numerical aperture × 1000. The maximum useful magnification of the microscope is 130000.

3. Experimental section
Researchers of natural and climatic conditions, snow cover and air atmosphere, polygonal climate tests, as biological objects that cause bioinfection of polymer composites operated in open ecosystems. under the influence of abiogenic and biogenic factors at low positive and extremely low ambient temperatures.

3.1. Brief physical and geographical characteristics of the research area
The city of Yakutsk is located in the central part of the Republic of Sakha (Yakutia), in the Tuymaada valley, on the left bank of the Lena River, in its middle course, several northern parallels 62 degrees north latitude (coordinates: 62 ° 01′38 ″ N 129 ° 43′55 ″ E (G) (O) (I)), during which a long period of “white nights” occurs, and in winter (December), daylight hours last only 3-4 hours. Area - 122 km².

The city of Yakutsk is located in the permafrost distribution zone with a thickness of 200–1500 m.

In the area of the city, the thickness of permafrost rocks is 200–250 m, seasonally thawed layer is 2.5–3 m, and the temperature at a depth of 10 m ranges from -2 -8 ° С.

The climate is sharply continental with little annual rainfall.

The annual amplitude of Yakutsk is one of the largest on the planet, approximately equal to the annual amplitude of the “cold poles” of Oymyakon and Verkhoyansk, and the maximum temperature is 100 ° C (102.8 ° C).

3.2. The results of microbiological studies of soils and soils
The total number of microorganisms in permafrost soils of the city of Yakutsk with a constant anthropogenic load in the upper horizons is not more than 100 thousand cells per gram of soil, which is slightly less susceptible to anthropogenic pollution than in the soil of Central Yakutia (table 1). An analysis of the structure of microbial communities showed that in the soil of the urban environment, bacterial forms dominate over fungal forms.

Among bacteria, spore forms (Clostridium, Bacillus) prevail over the non-spore group (Escherichia, Citrobacter, Serratia, Pseudomonas).

The total number of bacteria is at least 10 million cells per gram of dry soil, which is consistent with previous studies in this area [1-5].

In physiological composition, the isolated microorganisms differ in different types of permafrost soils.

In peat and sod-carbonate-podzolic soils, fungi of the genus Aspergillus, yeast of the genus Candida, and spore-forming bacteria of the genus Bacillus predominate; in loams, Pseudomonas and Bacillus are most common; In humus-gley and humus-gley tundra soils, psychrophilic microscopic fungi Aspergillus, Trichoderma, Alternaria and nocardia-like bacteria Nocardia and Streptomyces dominate, which also corresponds to the results of previous studies in this area [6]. At the next stage, soil samples taken from the territory of the climatic testing ground were studied as a factor of biological impact on PCM in the natural and climatic conditions of Yakutia.

The type of soil is sandy loam, light in mechanical composition.
Contains about 8-9% clay particles.
The plasticity number is 5%.
The quality of sand grains is fine sand.
As a background sample, soil samples were taken 3.7 km from the landfill (Yakutsk, Sosnovy Bor, Sergelyakhskoye highway 12 km).

Soils were selected in such a way that the type of soil corresponded or was approximate with the type of soil in the polygonclimatic test (Yakutsk, Avtodorozhnaya St., 20).
Table 1. Comparative characteristics of soils of Central Yakutia.

| Place of selection                                      | The total number of microorganisms, cl/g | pH    | Soil moisture, % | MPC excess, mg/kg  |
|--------------------------------------------------------|------------------------------------------|-------|------------------|--------------------|
| Megino-Kangalass district, shore of the lake Okunevo    | 31,0                                     | 7,5   | 33,2             | Does not exceed    |
| Nam district, Earl coast                                | 1,0                                      | 8,0   | 20,3             | P₂O₅ – 434,3       |
| Khangalass district, Bulgunjakhtakh                     | 170,0                                    | 8,2   | 34,6             | Does not exceed    |
| Khangalass district, Bulgunjakhtakh, Lena riverbank     | 0,2                                      | 7,7   | 36,3             | P₂O₅ – 416,5       |
| Khangalass district, Bulgunnyakhtakh                    | 0,001                                    | 6,9   | 26,2             | P₂O₅ – 354,4       |
| Khangalass district, V. Bestyakh                         | 26,0                                     | 7,3   | 31,1             | Does not exceed    |
| Amga district, industrial zone Hoiuu                    | 0,001                                    | 8,2   | 18,3             | Cl – 202,5         |
| Yakutsk, st. Lermontov                                  | 9,5                                      | 8,4   | 21,0             | Cu – 3,0           |
| Yakutsk, Pokrovsk road                                  | 3,6                                      | 7,3   | 28,4             | P₂O₅ – 435,2       |
| Yakutsk, Vilyui road                                    | 4,5                                      | 8,0   | 23,9             | Cu – 3,2           |
| Yakutsk, st. Dzerzhinsky                                | 10,0                                     | 8,2   | 19,2             | Does not exceed    |

The results of studies of the physiological groups of microorganisms in soil samples taken from the territory of the climatic test site testified to the dominance of bacterial forms over microscopic fungi and microflora of the actinomycete line (microorganisms combining the properties of bacteria and molds) (table 2).

Table 2. The total number of heterotrophic groups of microorganisms in soils, million CFU / g dry soil.

| No catalog samples | Bacteria | Spore-forming bacteria | Fungi | pH    | Moisture, % |
|--------------------|----------|------------------------|-------|-------|-------------|
| PKI -1             | 227,63±5,17 | 3,97±6,57               | 116,50±4,30 | 8,08 | 11,70       |
| PKI -2             | 109,67±2,48 | 3,37±0,00               | 15,10±6,57  | 8,12 | 11,10       |
| PKI -3             | 108,10±2,48 | 0,00±0,00               | 40,40±2,48  | 8,06 | 11,20       |
| PKI -4             | 280,32±8,96 | 4,91±0,00               | 24,80±6,25  | 8,07 | 8,50        |
| PKI -5             | 432,88±4,30 | 3,35±0,00               | 23,10±4,97  | 8,12 | 10,60       |
| Background         | 305,52±6,57 | 6,90±2,48               | 61,10±2,48  | 7,69 | 13,10       |

The dominance in the soil samples of bacterial microflora, the family of Enterobacteriaceae, over fungal conditions determine that fungal forms develop better on moist substrates with a moisture content of more than 60-70% and prefer acidified warm substrates (pH 3.0-5.5).

At that time, as at the time of selection, soil soils were identified as weakly alkaline, slightly moistened.

The ambient temperature in Yakutsk, during the growing season (May-September), averages 21.6 ° C, on the day of sampling, (September 11) +8 °C, soil temperature in depth, the occurrence of roots of herbaceous plants on the day of sampling is + 4 °C, which is not enough for the development of the most common mesophilic groups of soil microorganisms. The composition of soil fungi is different. So, yeast of the genus Candida, mold fungi of the genera Penicillium, Trichoderma, Fusarium were isolated from a sample of the background soil. Low saprophytic fungi of the genera Ulocladium,
Penicillium and pathogenic species Aspergillus (A. niger, A. fumigatus, A. flavus) were isolated from the soil of the climate test site. The studied areas are assessed as enriched.

The mineralization coefficient in the soil, climatic tests are higher than in the background soil (table 3).

**Table 3.** The number of microorganisms that assimilate various forms of nitrogen, million CFU / g dry soil.

| No. catalog samples | Mineral forms of nitrogen | Organic forms of nitrogen | Mineralization coefficient, % |
|---------------------|---------------------------|---------------------------|-------------------------------|
| PKI -1              | 227,63±5,17               | 3,97±6,57                 | 116,50±4,30                   |
| PKI -2              | 109,67±2,48               | 3,37±0,00                 | 15,10±6,57                    |
| PKI -3              | 108,10±2,48               | 0,00±0,00                 | 40,40±2,48                    |
| PKI -4              | 280,32±8,96               | 4,91±0,00                 | 24,80±6,25                    |
| PKI -5              | 432,88±4,30               | 3,35±0,00                 | 23,10±4,97                    |
| Background          | 305,52±6,57               | 6,90±2,48                 | 61,10±2,48                    |

The number of bacteria of the actinomycete-nocardioform line (dominants of Rhodococcus and Streptomyces) is not high. Microorganisms oxidizing sulfur and iron are not isolated (table 4).

**Table 4.** The content in the soil samples of physiological groups of microorganisms, million CFU / g dry soil.

| No. catalog samples | Nitrificators | Denitrifiers | Serobacteria | Iron bacteria | Actinomycetes |
|---------------------|---------------|--------------|--------------|---------------|---------------|
| PKI-1               | 0,02±2,28     | 0,01±2,48    | 0,00         | 0,00          | 0,02±2,28     |
| PKI-2               | 0,02±2,28     | 0,01±2,48    | 0,00         | 0,00          | 0,02±2,48     |
| PKI-3               | 0,02±2,28     | 0,02±2,48    | 0,00         | 0,00          | 0,03±2,48     |
| PKI-4               | 0,01±2,28     | 0,01±2,48    | 0,00         | 0,00          | 0,02±2,48     |
| PKI-5               | 0,03±2,48     | 0,01±2,48    | 0,00         | 0,00          | 0,02±2,48     |
| Background          | 0,04±2,48     | 0,03±2,48    | 0,00         | 0,00          | 0,06±2,48     |

According to sanitary and bacteriological indicators, the studied soil samples are assessed as moderately contaminated (table 5).

**Table 5.** The results of the sanitary-bacteriological assessment of soils, Index, 1.0 g.

| No. catalog samples | Pathogenic, including Salmonella | Coliform bacteria | Enterococcus | Clostridium perfringens |
|---------------------|---------------------------------|-------------------|--------------|-------------------------|
| PKI-1               | 0,0                             | 1,0               | 1,0          | 0,0                     |
| PKI-2               | 0,0                             | 1,0               | 1,0          | 0,0                     |
| PKI-3               | 0,0                             | 1,0               | 1,0          | 0,0                     |
| PKI-4               | 0,0                             | 1,0               | 1,0          | 0,0                     |
| PKI-5               | 0,0                             | 1,0               | 1,0          | 0,0                     |
| Background          | 0,0                             | 1,0               | 1,0          | 0,0                     |

The main differential diagnostic properties of dominant microorganisms are presented in table 6. Thus, according to the results of microbiological studies, we can conclude that the biological activity of the soil of the climate test site at the time of sampling (September 11, 2018) was reduced, and the high number of pathogenic species of molds (on average 43.98 million cells per 1 g of absolutely dry soil weight) may well be the cause of bio-contamination of atmospheric air and materials in contact with the soil.
### Table 6. The main differential diagnostic properties of dominant microorganisms isolated from the soil of the climate test site.

| The properties                  | Bacillus vulgarus | Proteus vulgarus | Pseudomonas aeruginosa | Aspergillus fumigatus | Penicillium chrysogenum | Aspergillus niger |
|--------------------------------|------------------|------------------|------------------------|-----------------------|--------------------------|-------------------|
| Oxidase                        | +                | -                | -                      | -                     | -                        | -                 |
| Catalase Production            | +                | +                | -                      | -                     | -                        | -                 |
| Gelatinase Liquefaction        | +                | +                | -                      | -                     | +                        | -                 |
| Lecithinase formation          | -                | +                | +                      | +                     | -                        | +                 |
| Hydrolysis of starch           | -                | -                | +                      | +                     | +                        | -                 |
| Acid from: glucose             | -                | +                | -                      | +                     | +                        | +                 |
| lactose                        | -                | -                | +                      | +                     | +                        | -                 |
| maltose                        | -                | -                | -                      | +                     | +                        | +                 |
| mannitol                       | -                | -                | -                      | +                     | +                        | -                 |
| xylose                         | -                | -                | -                      | +                     | +                        | -                 |
| glycerin                       | -                | -                | -                      | +                     | -                        | -                 |
| arabinose                      | -                | -                | -                      | +                     | -                        | -                 |
| sorbitol                       | +                | -                | +                      | +                     | +                        | -                 |
| sucrose                        | -                | +                | -                      | +                     | +                        | +                 |
| Sodium Citrate Disposal        | -                | -                | +                      | +                     | +                        | -                 |
| Disposal of sodium             | -                | -                | +                      | +                     | +                        | -                 |
| malonate                       | -                | -                | -                      | +                     | -                        | -                 |
| Urease formation               | -                | +                | +                      | +                     | -                        | -                 |
| Indole formation               | -                | +                | -                      | -                     | -                        | -                 |
| Hydrogen sulfide               | -                | +                | -                      | -                     | -                        | -                 |
| Lysine                         | -                | -                | -                      | -                     | -                        | -                 |
| Ornithine                      | +                | -                | -                      | -                     | -                        | -                 |

*Legend: + test positive; - test is negative; (+) weakly positive test*

3.3. *The results of a microbiological study of atmospheric air*

Currently, standards for sanitary-microbiological air assessment are developed only for enclosed spaces. For open ecosystems, there are no such standards yet.

Existing GOST 17.2.3.01-86 and RD 52.04.667-2005 provide only a study on the concentration in the air of suspended solids, sulfur dioxide, carbon monoxide, nitric oxide, nitrogen dioxide, benz (a) pyrene, formaldehyde, ammonia.

To study the influence of environmental factors on the activation of PCM bio-contamination, atmospheric air was studied on the presence of resident (autochthonous) microflora, which is mainly formed due to soil microorganisms.

On the day of sampling, September 11, cloudy weather was observed, air temperature during the day +8 °C, north wind 2 m / s, atmospheric pressure 752 mm Hg. Air samples were taken by aspiration on Saburo medium and meat-peptone agar (MPA).

In the landscape, isolated microflora from atmospheric air, mold fungi of the genera *Penicillium, Rhizopus* and *Aspergillus*, and yeast of the genus *Rhodotorula* dominated.

Bacterial microflora is represented by pigment-forming micrococci of the genus *Kocuria*, spore-forming bacilli (*Bacillus*), non-fermenting bacteria of the genus *Chryseobacterium* (*Flavobacterium*). The main differential diagnostic properties of microorganisms isolated from the atmospheric air of the territory of the climate test site are presented in table 7.
Thus, according to the results of microbiological studies, we can conclude that the atmospheric air in the area of the climate test site at the time of sampling contained various types of microorganisms, including pathogenic species of *Aspergillus* (*A. niger*) molds, which may well serve biological contamination agents of the surface soil layer, environmental objects and materials in contact with soil and air.

As a result of further research, the contamination of finished products and swabs with PCMs was established, located at the climate test site by microorganisms of various taxonomic accessories.

### 3.4. The results of microbiological studies of PCM samples
In the framework of this work, studies were performed on the microbial contamination of finished PCMs. A total of 38 experimental PCM samples were examined, exhibited at the climatic test site in the city of Yakutsk.

A wide variety of microorganisms was isolated from PCM fragments and washes from their surface; the most frequently isolated were spore-forming bacteria of the genus *Bacillus*, yeast-like
fungi of the genera *Candida*, *Rhodotorula*, and mold fungi of the genera *Penicillium*, *Aspergillus*, *Fusarium* and *Trichoderma*.

4. Conclusion
A wide variety of microorganisms was isolated from environmental objects and PCM fragments.

It has been established that the soil cover and atmospheric air in the area of the climate test site (Yakutsk, Avtodorozhnaya St., 20) contain various types of microorganisms, including pathogenic species of mold fungi of the genus *Aspergillus* (*A. niger*; *A. fumigatus*), which most likely they could serve as PCM bio-contamination agents in contact with soil and atmospheric air in the territory of the climatic test site. What is confirmed by the results of microbiological studies. Of the total biodiversity of microflora isolated from experimental PCM specimens exhibited at the climatic test site, fungi of the genus *Aspergillus* accounted for 38.3%, of which the dominant ones were *A. niger* (52.3%) and *A. fumigates* (23.8%).

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