

Toward protection of urban stone structures from decay using pleurotus ostreatus: mycokarst self-healing material

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Abstract. The ongoing decay of urban stone structures creates a need for smart materials that enhance durability over the life cycle. Biotechnologies based on fungus *Pleurotus ostreatus* are explored as a method to strengthen substrates prone to destruction in this study. Their ability to alter properties and adapt to extreme conditions provides the basis for self-healing, strong, resilient, and environmentally friendly biocomposites. Some fungal strains are known to precipitate calcium carbonate (CaCO_3) and to heal cracks in concrete, while *Pleurotus ostreatus* stimulates calcium oxalate (CaOx) formation. The study investigates whether these microorganisms are able to facilitate carbonate biomineralization without compromising their ability to act as natural sealants. The resulting Mycokarst material, formed from CaCO_3 -based karst soil, rice, dolomite flour, and *Pleurotus ostreatus* mycelium, demonstrated cyclic self-healing and strength gain. It promotes self-strengthening of weak karst soils and stone structures through the formation of mycelial and limestone frameworks without external intervention. Mycokarst represents a new generation of smart biocomposites and offers a green approach to protecting urban mineral-based infrastructure from decay, while reducing CO_2 emissions and environmental impact and minimizing human and technological interference with construction and restoration.

Keywords: biocomposite, carbonate biomineralization, fungal mycelium, karst soil, mycelial and limestone frameworks, Mycokarst, *Pleurotus ostreatus*, self-healing, urban stone structures

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1. INTRODUCTION

As the natural landscape and built heritage in urban environments, stone structures are exposed to constant physical, chemical, and biogenic weathering throughout their service life. Temperature fluctuations between winter and summer, day and night cause materials to contract in cold periods and expand in heat, leading to cracks formation [1-3].

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Weak karst soils (dolomite, limestone) are vulnerable to water erosion and landslides and are a key factor in unpredictable destruction of infrastructure and settlements. Decay is accelerated by anthropogenic factors and climate variability [4]. In this regard, there is a growing need for smart materials that can controllably change properties during operation, reducing matrix permeability and increasing functional efficiency of urban structures [5-7].

This study focuses on biotechnology based on *Pleurotus ostreatus* as an approach to strengthen materials with a high potential for destruction, leveraging the fungus' resistance to external factors and its adaptability to extreme conditions. In particular, fungal mycelium can act as a natural sealant and replace synthetic binding polymers and a basis for self-healing, ultra-strong, and environmentally friendly building materials depending on processing methods [8-9]. Being growing on organic substrates, such as agricultural waste, it can yield durable biocomposites after drying [9-12]. For example, *Pleurotus ostreatus* and *Ganoderma lucidum* mycelium substrates grown on rice and wheat husks or sawdust and later heat-treated can be comparable to concrete in strength while remaining lightweight and biodegradable. It was found flexural and compressive strength exceed those of existing materials by 2-6 times [11-12].

Other studies explore self-growing and self-healing biocomposites based on *Pleurotus ostreatus* using nutrient bases such as coffee grounds, agar gel, malt, and peptone, with 3D printing as a production method. These materials demonstrate improved elastic modulus in 2 times within 28 days and self-healing capabilities [13-14]. Brown rice and potato dextrose agar also promote the growth of *Pleurotus ostreatus* mycelium, with the diameter of its formation reaching 8.88 ± 0.12 cm within 10 days [15].

Some fungal strains are known to precipitate calcium carbonate (CaCO_3) and to heal cracks in concrete, though the specific role of *Pleurotus ostreatus* requires further study [16-22]. It was established that calcium sources such as agricultural lime, eggshells, and seashells can enhance *Pleurotus ostreatus*-based substrate yield and stimulate calcium oxalate (CaOx) formation [23-27].

The current research investigates whether *Pleurotus ostreatus* is able to produce calcium carbonate (CaCO_3) and form a limestone framework without compromising mycelial ability to be a natural sealant. The goal is to develop the Mycokarst self-healing material based on biosubstrate with calcium sources, nutrients, and *Pleurotus ostreatus* mycelium that promotes self-strengthening of weak karst soils and stone structures. Soil influenced by groundwater is considered hypothetically as a natural substrate that supports mycelial growth and necessary moisture. While detailed methods for applying Mycokarst to existing stone structures for their self-healing are beyond the scope of this article, potential applications are discussed.

2. METHODS AND MATERIALS

2.1. Materials and equipment

To achieve the research objectives, materials were organized into three groups: agents, substrate matrix (base), mineral and calcium supplements, as well as moisture-retaining and gelling additives (Table 1). Additional consumables and equipment were prepared (Table 2).

Table 1. Biosubstrate materials.

№	Material name	Properties and role
	Agents	
1	<i>Pleurotus ostreatus</i> mycelium, M5 strain	Natural reinforcement basis, a catalyst for calcium carbonate precipitation and biomineralization. It is utilized for cultivating and creating high-strength biocomposites
	Substrate matrix (base)	
2	Soil	Basis for moisture retention and aeration, nutrients distribution and mycelial growth, calcium source
3	Karst soil	Existing material for strengthening by <i>Pleurotus ostreatus</i> mycelium, calcium source

Continuation of Table 1

4	Distilled water	Medium that provides substrate moisture and mycelium development
	Mineral and calcium supplements	
5	Dolomite flour	CaMg(CO ₃) ₂ , source of calcium (30-35%) and magnesium (20-25%) providing pH 7-9 that is favorable for both carbonate biomineralization and mycelium development
6	Rice flour	Source of silicon, B vitamins, carbohydrates (starch) that provides rapid colonization of the substrate by mycelium
7	Yeast extract	Rich source of B vitamins, potassium, amino acids, and nitrogen for the mycelium development
8	Slaked lime	Calcium source that simulates karst conditions for carbonate biomineralization
	Moisture-retaining and gelling additives	
9	Xanthan gum	Gelling agent to retain moisture in substrates and stabilize suspensions

Table 2. Consumables and equipment.

№	Material name	Properties and role
1	Containers	Mixing substrate components
2	Thick polyethylene bags 15*10	Growing substrates
3	Polyethylene film	Maintaining substrate moisture and limiting oxygen access
4	9 cm-diameter Petri dishes	Mycelium growth in suspensions
5	Gloves	Sterilization
6	Alcohol solution	Sterilization
7	Tekhmetr KVO-7 — pH, moisture and illumination meter	Monitoring process parameters
8	Sierra Moderna — electronic scales	Measuring samples weight loss

2.2. Methods

The empirical study was conducted using observation, comparison, measurement, and experimentation and consisted of three stages, the goals and methodologies of which are described below.

2.2.1. Experiment 1. Establishing the ability of *Pleurotus ostreatus* fungal mycelium to grow in nutrient suspensions with and without calcium sources

The goal is to determine the ability of *Pleurotus ostreatus* mycelium to grow in aqueous environments with and without calcium sources, as well as the rate of its linear growth and minerals formation.

1. Preparation of suspensions based on water, rice flour (R), yeast (Y), and xanthan gum (G) with calcium sources (D, L) and without them:

- water 500 ml, rice flour 30 g, yeast 5 g, xanthan gum 3 g (RGY);
- water 500 ml, rice flour 20 g, yeast 5 g, dolomite flour 10 g, xanthan gum 3 g (RGYD);
- water 500 ml, rice flour 20 g, yeast 5 g, 10 g slaked lime, xanthan gum 3 g (RGYL);
- water 500 ml, rice flour 20 g, yeast 5 g, dolomite flour 10 g, slaked lime 10 g, xanthan gum 3 g (RGYDL).

2. The components are mixed to homogeneous suspensions in sterilized containers.

3. The suspensions are distributed into four sterilized Petri dishes.

4. *Pleurotus ostreatus* mycelium grains (PO) are added on top of each suspension.

5. The suspensions are incubated for 14 days under aseptic conditions at 20°C in a semi-dark environment (10 lux).

6. Results are recorded daily for two weeks.

2.2.2. Experiment 2. Establishing the ability of *Pleurotus ostreatus* fungal mycelium to grow in solid soil substrates with and without the nutrients and calcium sources

The goal is to determine the spatial growth rate of *Pleurotus ostreatus* mycelium in soil-based substrates with and without nutrients and calcium sources, as well as its capability of carbonate biomineralization with substrates hardness changes monitoring.

1. Boiling water is poured over the soil in a 1:1 ratio to remove competing microbes.
2. The soil should cool for about 5 hours until 80-90% humidity level.
3. Preparation of substrates based on soil (S) and *Pleurotus ostreatus* mycelium (PO) with nutrients (R, D) and without them:
 - soil 100 g, mycelium of *Pleurotus ostreatus* 10 g (control) (SPO);
 - soil 100 g, *Pleurotus ostreatus* mycelium 10 g, dolomite flour 20 g (SPOD);
 - soil 100 g, *Pleurotus ostreatus* mycelium 10 g, rice flour 20 g (SPOR);
 - soil 100 g, *Pleurotus ostreatus* mycelium 10 g, dolomite flour 10 g, rice flour 10 g (SPORD).
4. The components are mixed to homogeneous substrates of 70% humidity level in sterilized containers. If it is necessary distilled water can be added to achieve it.
5. The substrates are distributed into sterilized bags. It is necessary to make 2-3 cuts (0.5-1 cm) to allow oxygen access.
6. The substrates are incubated for 14 days under aseptic conditions at 20°C in a semi-dark environment (10 lux).
7. Results are recorded daily for two weeks.

2.2.3. Experiment 3. Establishing the ability of *Pleurotus ostreatus* fungal mycelium to promote self-healing of karst soil composites with Mycokarst material formation

The goal is to determine the ability of *Pleurotus ostreatus* mycelium to promote calcium carbonate biomineralization in a karst soil-based substrate and form self-healing and self-strengthening biocomposites with mycelial and limestone frameworks.

1. Boiling water is poured over the soil in a 1:1 ratio to remove competing microbes.
2. The soil should cool for about 5 hours until 80-90% humidity level.
3. Preparation of a substrate with the following components: soil 1000 g, *Pleurotus ostreatus* mycelium 200 g, rice flour 200 g, dolomite flour 200 g (SPORD).
4. The substrate is grown for 90 days: first month in aseptic conditions at 15-22°C in a semi-dark environment (10 lux), then maintaining in self-preservation without external impacts, temperature and humidity changes.
5. Results are recorded on days 1, 3, 7, 14, 21 and 28 of the first month, then once a week.

3. RESULTS AND DISCUSSION

3.1. Experiment 1 results

Mycelial growth rate and diameter: in suspensions 1 (RGY) and 2 (RGYD), growth began on day 3. It accelerated to day 5, reaching diameters of 4.5 cm and 7.8 cm, respectively. In the suspension 1 (RGY), mycelial growth ceased after day 5 and remained stable with minor edge fragmentation. In suspension 2 (RGYD), growth continued on day 7, reaching a maximum diameter of 9 cm (a Petri dish was completely covered by mycelium). In suspension 4 (RGYDL), growth was rapid to day 7 to 7 cm, then slowed; maximum diameter of 9 cm was reached on day 11. In suspension 3 (RGYL), the mycelium did not develop until day 6, then slowly increased by 0.5–0.7 cm daily, reaching 4.3 cm on day 14 (Fig. 1, 2).

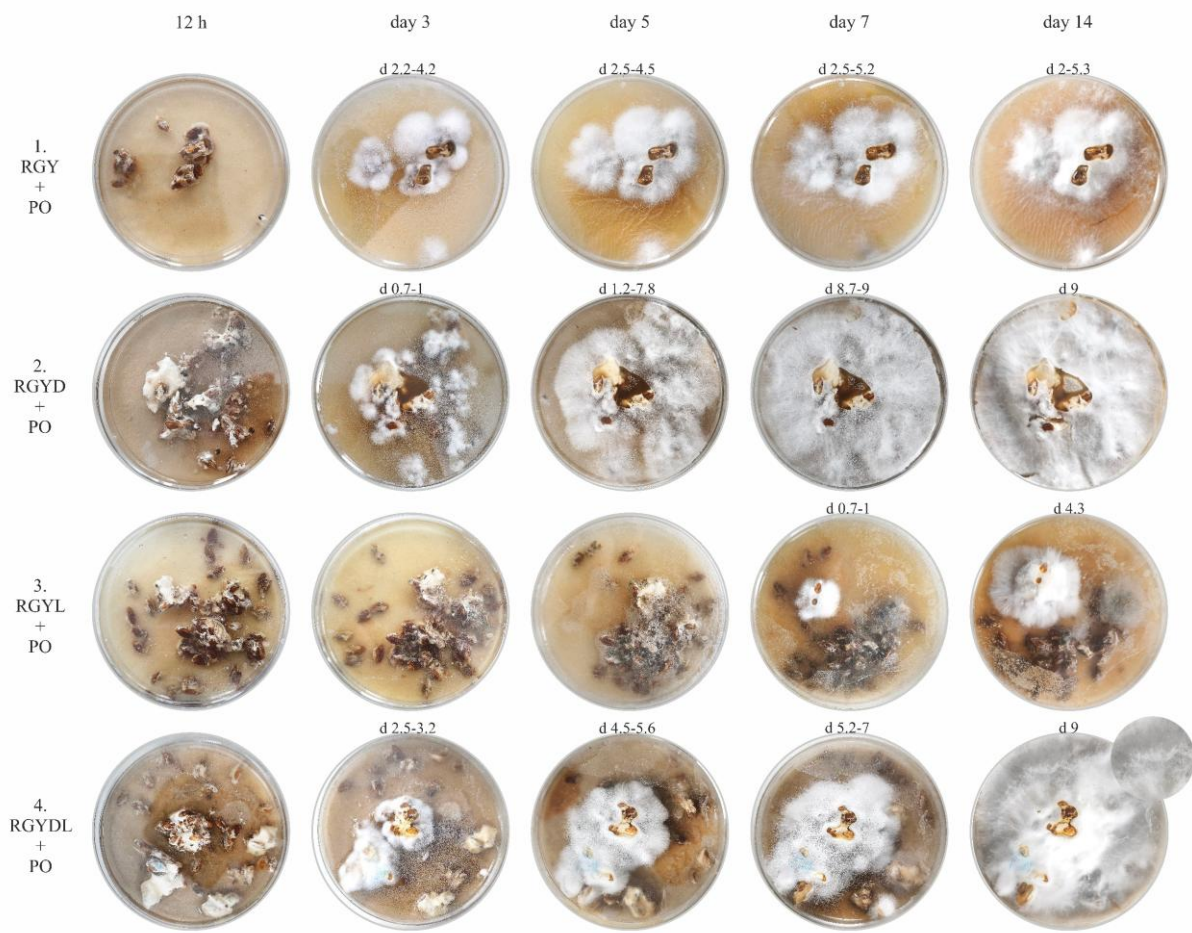


Fig. 1. The growth of *Pleurotus ostreatus* mycelium (PO) in nutrient suspensions based on water, rice flour (R), yeast (Y), and xanthan gum (G), with calcium sources – dolomite flour (D) and lime (L) – (RGYD, RGYL, RGYDL) and without them (RGY). Author's image.

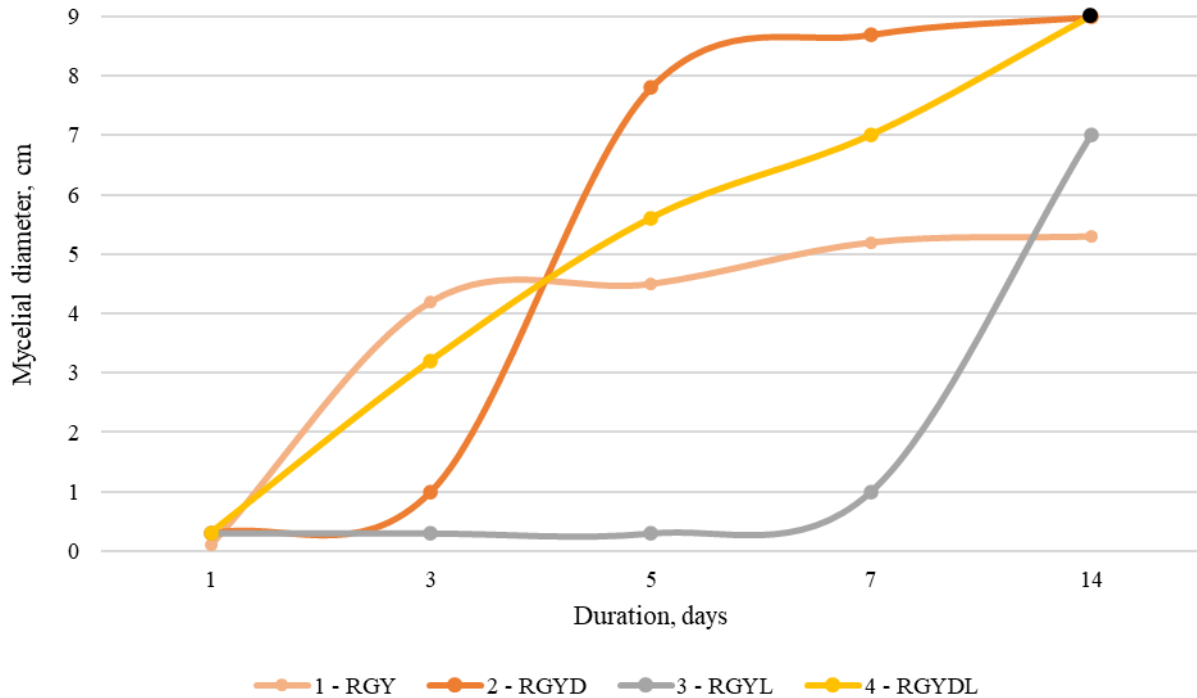


Fig. 2. Dynamics of *Pleurotus ostreatus* (PO) mycelial growth rate and diameter in suspensions 1-4. Author's image.

Mycelial density: although mycelium reached the maximum diameter in suspension 2 (RGYD), the formations were delicate and threadlike. In the suspension 1 (RGY), the formation was denser and foamy. Suspensions 3 (RGYL) and 4 (RGYDL) produced dense mycelium, particularly 3 (RGYL) with lime.

Weight loss of samples: weight loss of 2 ml per day was observed in the suspensions through water evaporation. However, the nutrients and gum allowed continued mycelial growth despite moisture depletion.

Calcium carbonate formation: calcite crystals about 0.5 mm formed along the dish edge near the mycelial boundary in substrate 4 (RGYDL) on day 12. No such crystals were observed in the other substrates.

Absence of pathologies: all the samples were free of mold or other pathologies.

Based on the experiment 1, it was concluded that lime reduces mycelial growth, while dolomite flour promotes it. Rice flour accelerates growth most effectively, dolomite flour best promotes expansion. Mixing rice and dolomite flour yields denser mycelial structures. Xanthan gum provides gelation, retaining vitamins and water in suspensions, supporting mycelial growth. However, xanthan gum is not essential in later experiments, because soil itself provides stabilization. Thus, the combination of rice and dolomite flour accelerates mycelial growth, lime increases density of mycelial formations and provides calcium carbonate ones.

3.2. Experiment 2 results

Mycelial growth rate and size: after 12 hours, a rapid colonization was identified in substrate 4 (SPORD) with 1–2 cm massive mycelial formations. In substrate 3 (SPOR), dense 0.7–1.2 cm formations were demonstrated, in substrate 2 (SPOD) – delicate 0.3–0.6 cm ones; in substrate 1 (SPO) – a single one about 0.5 cm. By day 3, mycelium had colonized substrates 3 (SPOR) and 4 (SPORD), substrate 2 (SPOD) was 2/3 colonized, substrate 1 (SPO) was half colonized. By day 7, all the substrates were completely covered by mycelium, with differing patterns (Fig. 3).

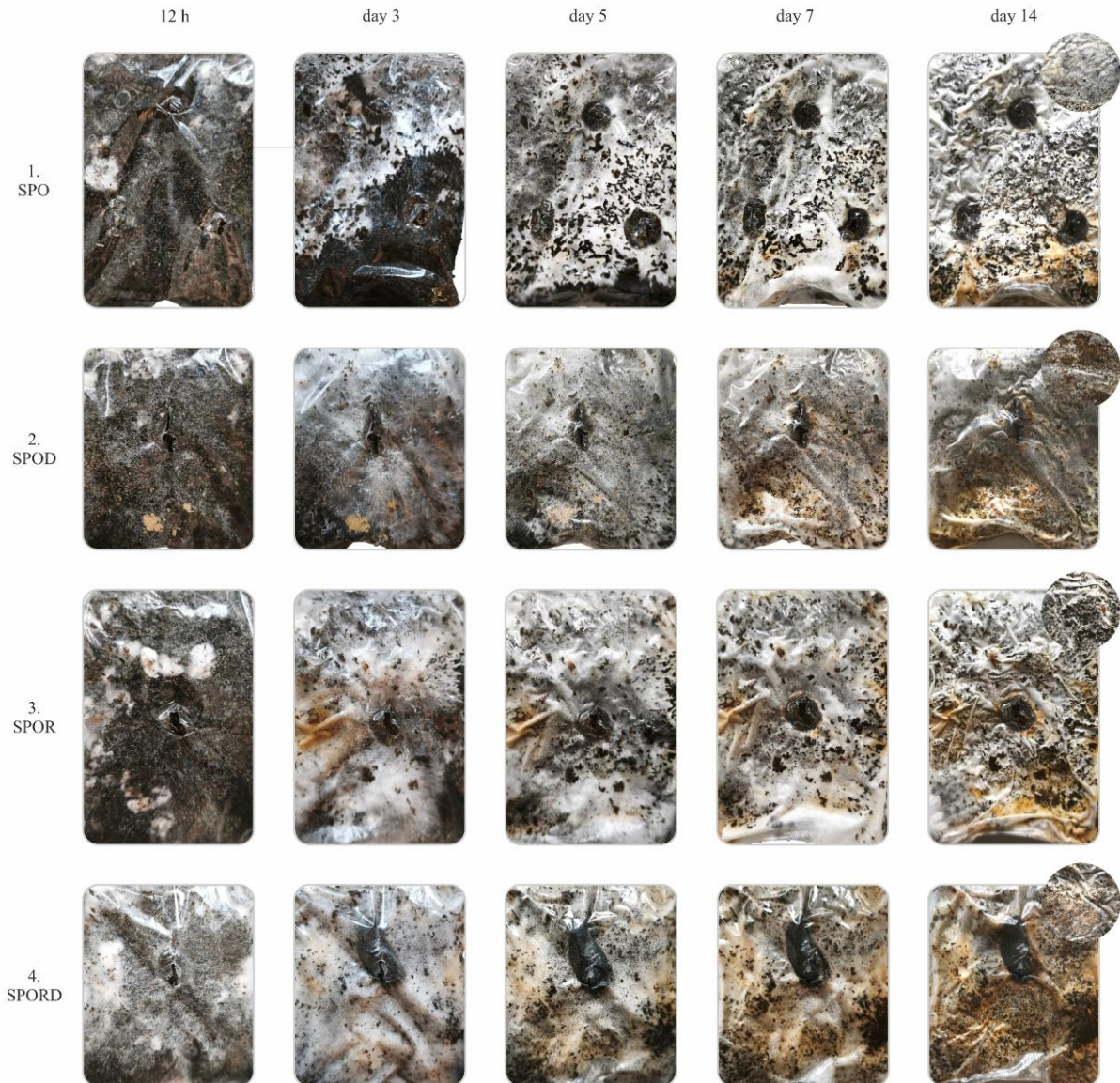


Fig. 3. The growth of *Pleurotus ostreatus* mycelium (PO) in solid soil substrates (S) with rice (R) and dolomite flour (D) (SPOD, SPOR, SPORD) and without them (SPO). Author's image.

Hardness of substrates: all the samples had similar hardness, but the dolomite-based substrate 2 (SPOD) was slightly ahead, followed by substrate 3 (SPOR), then – 4 (SPORD), and finally – 1 (SPO). Despite a dense white pattern in SPO substrate, it was noted lacked the necessary components to achieve hardness comparable to that of the dolomite-based one. The positive result demonstrates the ability of *Pleurotus ostreatus* mycelium to develop and form strong and elastic framework with minimal nutrients.

Weight loss of samples: the substrates weight loss ranged from 0.3–2 g per day in the first 5 days, averaging 2–3 g over 3 days. On day 14, substrate 2 (SPOD) lost the least weight (3 g), followed by substrate 3 (SPOR) and 4 (SPORD) (4 g), and then – 1 (SPO) (6 g). Weight stability for 2–3 days was observed in some substrates. It suggests ongoing mycelial growth even at low moisture, coinciding with increased substrate strength (Fig. 4).

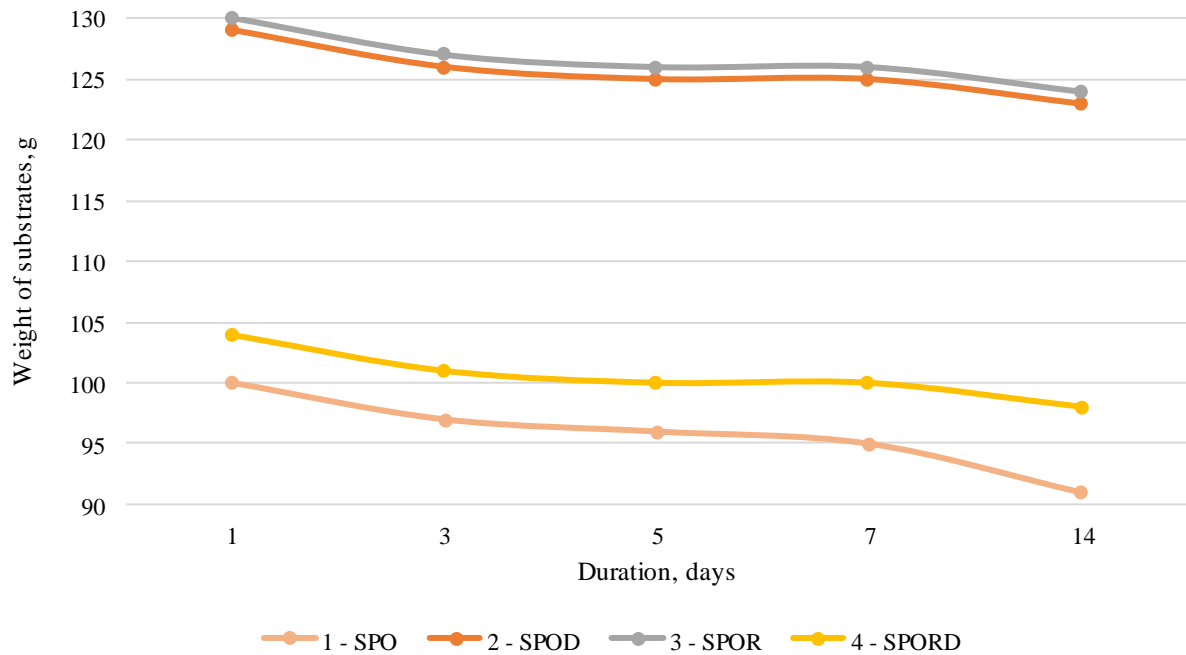


Fig. 4. Dynamics of substrates 1-4 weight loss. Author's image.

Calcium carbonate formation: on days 10-12, calcite crystals of 0.3-0.6 mm were observed on the back surface of the substrate with dolomite flour. However, it was difficult to distinguish carbonate from mycelial patterns as they formed a single white, elastic framework.

Absence of pathologies: all the samples were free of mold or other pathologies.

Based on the experiment 2, it was concluded that the combination of rice and dolomite flour supports strong mycelial development, results were superior to many literature reports in growth rate, size, and density. It was confirmed that the soil with dolomite flour mixture facilitates *Pleurotus ostreatus* mycelial growth and promotes alkaline conditions conducive to calcite precipitation.

Based on the experiments 1 and 2 results, it was decided to utilize a 1:1 combination of rice and dolomite flour for an experiment 3 on karst soil self-healing. The final stage aims to prove the hypothesis that lime within karst soil enhances denser framework formation and long-term strength gain. In this regard, it is necessary to supply nutrients for mycelial growth and to form natural reinforcement and limestone framework.

3.3. Experiment 3 results

Mycelial growth rate and size: as in the experiment 2, mycelial growth was observed within 12 hours. By day 3, the substrate was completely colonized with thin mycelial hyphae, by day 7 a dense white pattern covered a half of the substrate. Cyclical mycelial growth occurred within 90 days: rapid growth – from days 7-14, fruiting bodies were formed from days 14-21, a decline in growth – from days 21-28, followed by a second growth phase from days 42-70 with another fruiting event.

Mycelial and limestone frameworks: on day 90, the mycelium and limestone frameworks formed a single, hard and elastic white sample resembling a lightweight, durable brick (Fig. 5).

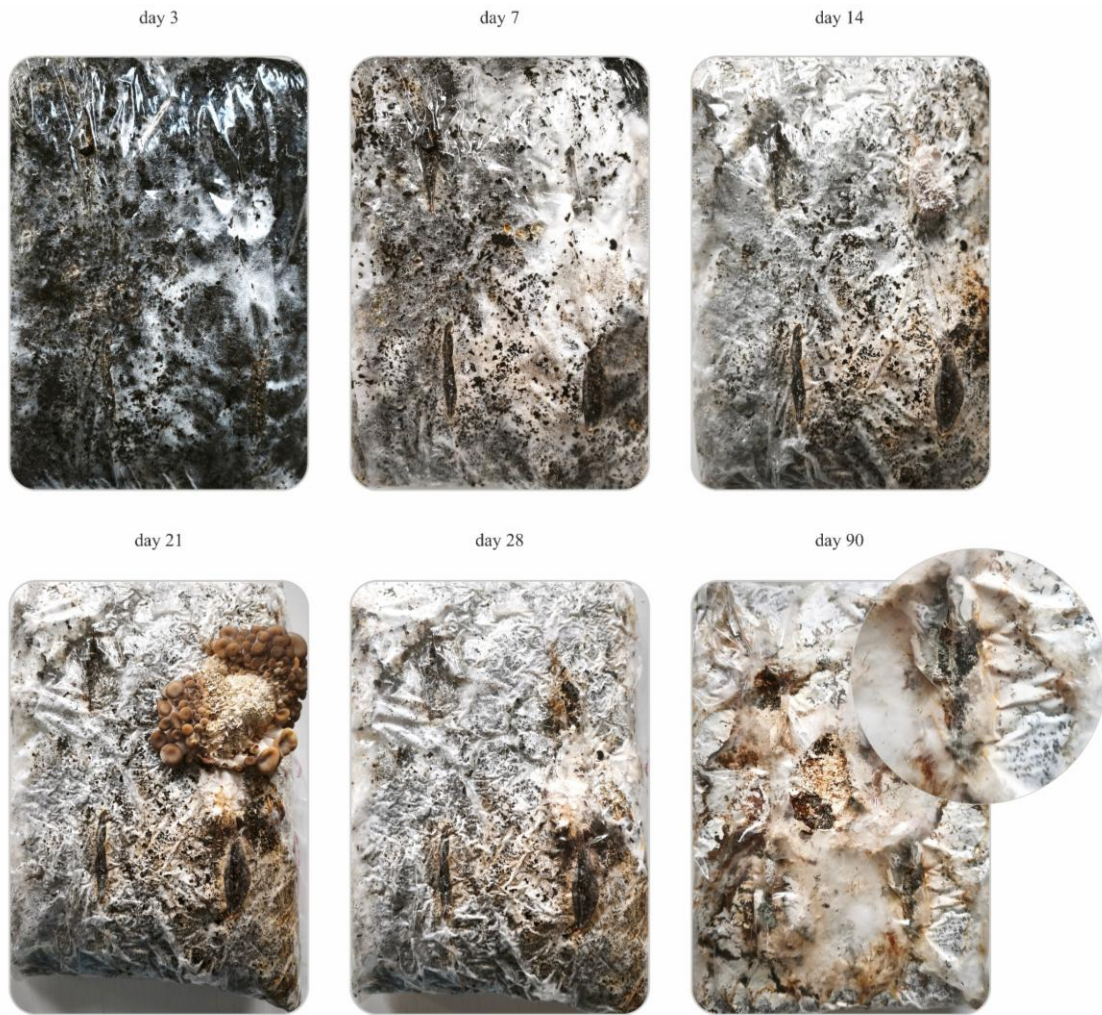


Fig. 5. Mycokarst self-healing composite material based on karst soil, rice and dolomite flour, and *Pleurotus ostreatus* mycelium: growth cycles with the mycelial and limestone frameworks formation. Author's image.

Samples strength: The strength reached a maximum of 1.1 MPa on days 20–21 and 70 (fruiting period). According to the scientific literature, drying a sample with heat treatment allows to increase strength 3–6 times. In this study, this process occurs without external intervention, as the goal was to extend the self-healing period while maintaining optimal strength and elasticity of the sample. As a result, strength decline after the first cycle was modest, suggesting the possibility of achieving high compressive strength and elasticity over several years (Fig. 6).

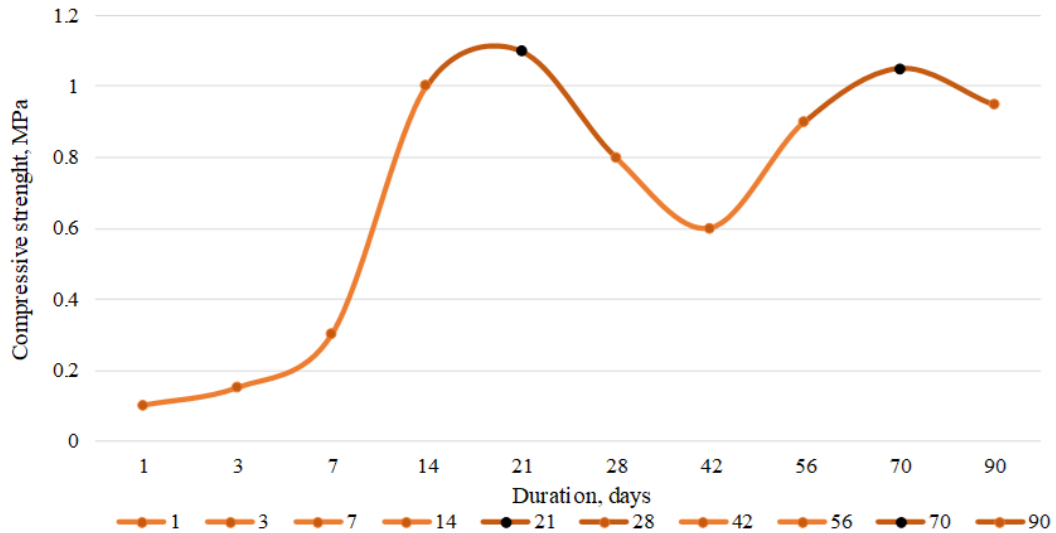


Fig. 6. Mycokarst self-healing material compressive strength within 90 days with growth cycles. Author's image.

Calcium carbonate formation: calcite microcrystals were observed on day 7, three days earlier than in the experiment 2. They increased in density over the ensuing days (most brightly on 7-14 days before fruiting). It was identified that in karst soil, crystals gradually form calcium carbonate minerals within a month and biomineralize into limestone within next months. On day 90, carbonate biomineralization was ongoing, and the pattern continued to evolve.

Absence of pathologies: the sample was free of mold or other pathologies.

The study results confirmed that the Mycokarst substrate based on karst soil, rice, dolomite flour and *Pleurotus ostreatus* mycelium is capable of self-healing. The process cyclical nature was established, despite the complete depletion of mineral nutrients and moisture in material over 90 days of experiment. The material evolves into a biocomposite that gains and maintains strength without external influence. The study scientific novelty is in the combined properties of *Pleurotus ostreatus* mycelium as a natural reinforcement, sealant, and a carbonate biomineralization agent forming limestone within stone materials, including CaCO_3 -based karst soil.

Taking the results into account, potential applications of Mycokarst have been identified, and the self-healing and reinforced samples are demonstrated (Fig. 7-10):

- Self-strengthening of karst and other mineral-based weak soils to protect human life and infrastructure from unpredictable destruction and collapses [8, 28] (Fig. 8);
- Restoration of existing buildings stone facades and internal structures to extend their life cycle and reduce maintenance, as well as preservation of cultural heritage [29] (Fig. 9);
- Construction of self-healing buildings and structures, while a partial substitution of cement binder with Mycokarst substrate will allow to reduce CO_2 emissions into the atmosphere [30] (Fig. 10).

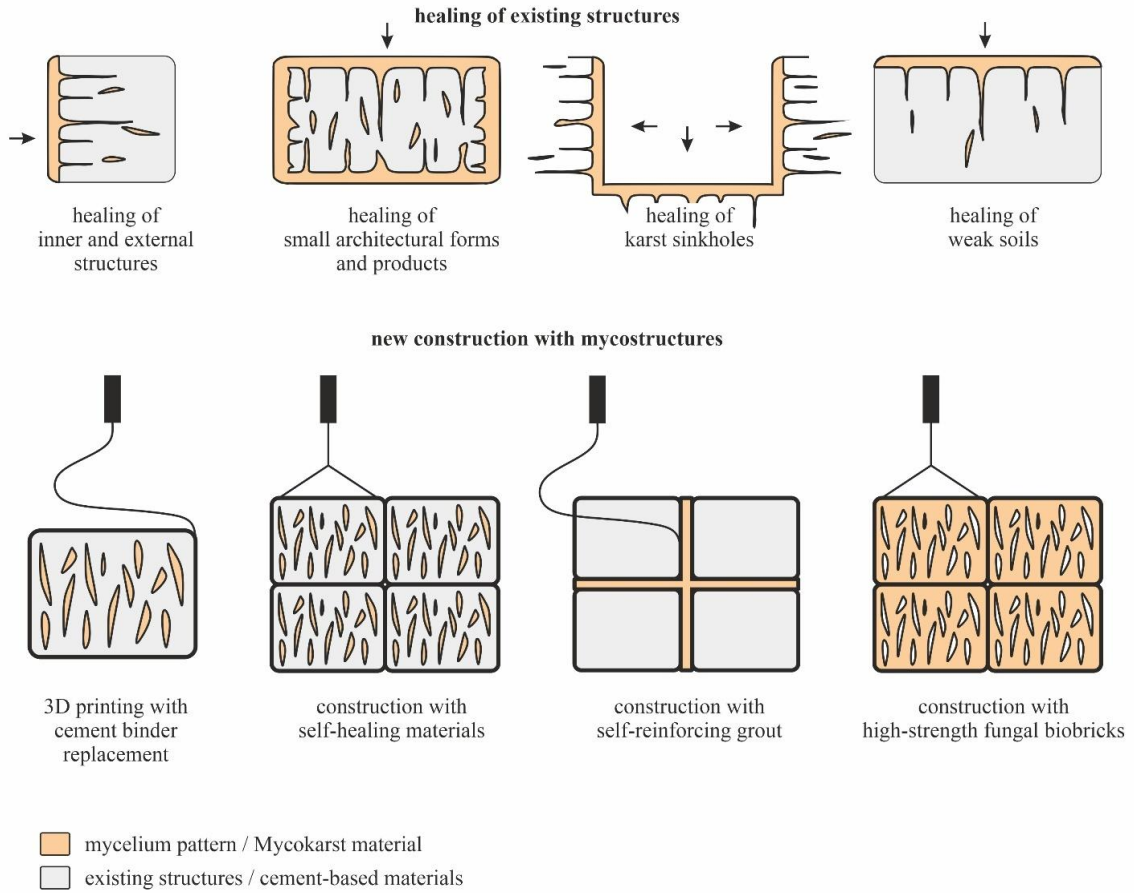


Fig. 7. Mycoarst self-healing material application areas diagram. Author's image.

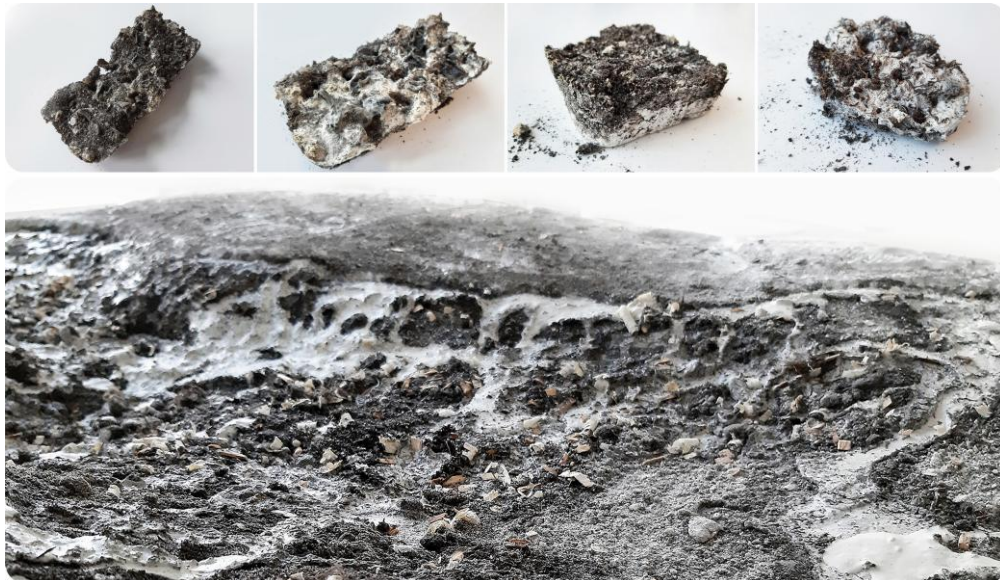


Fig. 8. The area of karst sinkhole limestone soil along the Noksa River in Kazan, healed and reinforced with Mycoarst material. Author's image.



Fig. 9. Elements of a historical mineral brick facade healed and reinforced with Mycokarst material. Author's image.



Fig. 10. 3D printed concrete structures elements, healed and reinforced with Mycokarst material. Author's image.

4. CONCLUSIONS

The present study demonstrates that biotechnology based on *Pleurotus ostreatus* provides a promising approach for strengthening mineral materials prone to destruction. The fungal mycelium exhibits metabolic and structural plasticity, enabling adaptation to extreme conditions and modification of substrate properties, thereby providing self-healing, durable, elastic, and environmentally friendly biocomposites.

Experimental results confirm the author's hypothesis that *Pleurotus ostreatus* not only stimulates calcium oxalate (CaOx) formation, but also promotes calcium carbonate (CaCO₃) precipitation and cracks healing in stone materials. This fact has not been described previously in the literature. It was found that the combination of rice and dolomite flour supports robust mycelial development and promotes alkaline conditions conducive to form natural reinforcement and limestone frameworks. The resulting Mycokarst material, consisting of CaCO₃-rich karst soil, rice, dolomite flour, and *Pleurotus ostreatus* mycelium, demonstrated cyclical self-healing and strength gains over 90 days of experimentation.

Mycokarst represents a new generation of smart biocomposites based on *Pleurotus ostreatus*, enabling self-strengthening of weak karst soils and stone structures without external intervention. The study's novelty lies in the integrated properties of *Pleurotus ostreatus* mycelium as a natural sealant, and an agent of carbonate biomineralization in the field of existing urban structures bioreinforcement. Mycokarst has potential for utilization for self-strengthening of karst and other mineral-based weak soils, restoration of existing buildings stone facades and internal structures to extend their life cycle and reduce maintenance, construction of self-healing buildings based on replacement of cement binder with natural ones.

Mycokarst is offered as a green approach to protecting urban mineral-based infrastructure from decay, while reducing CO₂ emissions and environmental impact and minimizing human and technological interference with construction and restoration. To advance practical application, future author's work should address scale-up trials, methodologies development with durability assessments under diverse climate conditions, and life-cycle and ecological impact analyses to define deployment limits and safety considerations.

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