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Water Absorption and Microbial Corrosion Resistance of Hemp Concrete Modified with the Acid Casein Admixture

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ABSTRACT

The hemp-lime composite is a material with high porosity; therefore, it has a high ability to absorb water. Long-term contact of the composite with water is a destructive factor for the material due to the content of organic components and the lack of a hydraulic binder. It is important to look for ways to modify the composition of the composite in order to reduce water absorption. In traditional old construction, acid casein was used to improve the strength and water resistance of lime mortars. In this study, the influence of the admixture of casein in the amount of 1, 3, 5% of the binder mass on the water absorption and capillary rise of the hemp-lime composite was examined. Both the content of shives and the admixture of animal origin may contribute to the development of biological corrosion, which is why microbiological tests were performed on composite samples using the impression and swab method. As the casein content increased, the water absorption of the composite was reduced. The rate of capillary rise and the amount of rising water were also reduced. The addition of casein limited the ability to absorb water without significantly increasing the susceptibility to the development of microorganisms. The research should be extended to include, among others: the influence of this admixture on the composite's vapor permeability and mechanical properties.

Keywords: hemp-lime, casein, water absorption, capillary rise, mold growth.

INTRODUCTION

Building materials have different susceptibility to mold growth. The criteria for the possibility of mold development include environmental conditions such as relative humidity and ambient temperature, as well as the period of exposure of the material to such environmental conditions [1–3]. The problem with building materials containing ingredients of organic origin, including plant origin, is susceptibility to biological corrosion. However, the hemp-lime composite, due to the high pH level of lime, around 12 [4], is highly resistant to mold development [5], however, the carbonation process lowers the pH level of the composite [6]. Walker et al. in work [5] they studied the development of microorganisms in hemp concrete. The samples were inoculated with a high concentration of cultures of microorganisms found in the air and soil, including: *Aspergillus* and *Penicillium* fungi and *Bacillus* bacteria. The authors showed that after 2 months the inoculated molds died due to lack of sufficient nutrients or unfavorable conditions, even though the humidity was maintained at 80% and the temperature was 30 °C. In work [6], three strains of bacteria found in hemp-lime composite were isolated and the composite was inoculated with suspensions containing these strains, and then the samples were seasoned at RH 98% and a temperature of 30 °C. Moreover, the authors showed the presence of mold on the composite

samples only 100 days after inoculation, because due to the carbonation process, the pH level was lowered to 8.7–9.2, creating more suitable conditions for the development of microorganisms. In the work [7], the possibility of mold development on hemp shives and a hemp-lime composite was examined at RH 75%, 85%, 95% and an ambient temperature of 15 °C and 23 °C. At 75% humidity, no microorganisms were observed, but it was also shown that lime had an inhibitory effect on the development of mold on hemp shives. In the work [8], the possibility of the development of mold fungi on the surface of a composite based on hemp shives and corn starch impregnated with linseed oil and tung tree oil was investigated. This investigation proved that microorganisms build colonies in deeper layers and on the surface of biocomposites. The dominant microorganisms were *Rh. oryzae, A*. f*umigatus*, and bacterium *P. putida*. In turn, in work [9], microbiological tests were performed on insulating boards based on hemp shives and starch, containing flame retardants. After six months of incubation, it was found that the dominant microorganism was *Rh. oryzae* and *A. fumigatus*. Moreover, in the works [8, 9] it was shown that the presence and activity of fungi in the material causes structural defects, such as a decrease in compressive strength.

The problem with making a hemp-lime mixture is its high need for mixing water to obtain the right consistency due to the high absorbency of hemp shives. After the first 10 minutes of immersion in water, their water absorption is about 95% [10] and after 48 hours, about 350% [11]. This is due to their high porosity, according to the literature, approximately 78% [12]. The characteristics of the shives influence the high water absorption of the hemp-lime composite. According to the literature [13], the absorbability of composites with a density of approximately 442–445 kg/m^3 is approximately 87–97% after 6 days of soaking in water, however, they contained an admixture of biopolymer that lowered the absorbability. Composites with a lower density of 404 kg/m^3, not containing hydrophobizing admixtures, are characterized by higher water absorption, approximately 138% [14]. In work [13], an admixture of gum arabic was used, which in the amount of 5% of the binder mass resulted in a reduction of water absorption by about 10% compared to the reference hemp-lime composite. In turn, in [15] a partial substitute for shives was used in the form of expanded perlite. Replacing 40% of the filler weight resulted in a reduction in water absorption by approximately 18%.

External walls are also exposed to water absorption due to capillary action. In some works, the susceptibility of hemp-lime composite to capillary rise was investigated [16–18]. Paulien de Bruijn et al. showed [19] that a composite based on hemp shives and a binder based on hydrated hydraulic lime and Portland cement with a density in the range of 587–733 kg/m^3 was characterized by a capillary rise coefficient of approximately 0.15 kg/m^2/s$^{0.5}$, and the water absorption rate was greater than in the case of ordinary concrete. However, they emphasized that the rate of evaporation of water accumulated in the material is also important. In the work [20] it was shown that 3% and 5% admixture of gum arabic reduced the height of the water rise by capillaries, i.e., by 10.3 and 21.0 %, respectively, after 8 days of testing, and slowed down the progress of capillary uptake in the hemp-lime composite. The work [17] showed that partial replacement of shives with expanded perlite (40%) decreased material absorbtivity (the total amount of absorbed water) but did not slow down the progress in the capillary rise phenomenon.

As indicated in the above paragraphs, many publications have addressed the problem of the susceptibility of lime-hemp composites to the development of biological corrosion associated with the growth of mold fungi. Various boundary conditions related to ambient temperature, relative humidity, exposure time, etc. were also taken into account. The influence of biological infection of the material on its properties was also examined. The above also presents numerous examples of research on the water absorption capacity of the composite. All studies emphasized that regardless of their composition, these composites are characterized by high water absorption due to their high porosity. However, there is a lack of this type of research on hemp-lime composites modified with acid casein, i.e. a biopolymer based on proteins of animal origin. Acid casein, combined with an alkaline solution (in these tests with calcium hydroxide), creates a waterproof substance with adhesive properties. This admixture was used in traditional construction as a modifier of lime mortars, improving their compressive strength [21]. Research [22–24] also showed an increase in the strength of sand and clayey soil-based building mixtures due to the addition of casein. Casein, as a protein, tends to create air bubbles during the mixture, which may change the pore distribution, e.g. lime binders [25]. Due to its high water resistance,

casein glue is used for gluing wood-based products [26, 27]. The waterproof properties of calcium caseinate, its effect on pore distribution, as well as the binding capacity of wood (in this case, shives) may have a beneficial effect on hempcrete during its contact with water, e.g. by limiting its water absorption and water transport by capillary action. On the other hand, an organic admixture may potentially contribute to the intensification of mold growth on the composite surface in unfavorable environmental conditions. Therefore, this is an issue worth investigating.

The research presented in this paper is a continuation of research on a lime binder modified with casein [25]. A novelty is the development of a hemp-lime composite with a variable amount of casein as an admixture intended to limit its water absorption and water transport by capillary action. The TDR (Time Domain Reflectomerty) method was used to measure and record capillary rise, which was not used for this type of composites in other studies, but only in our own earlier ones (composites with a different composition [17, 20]). Taking into account the sensitivity of the composite to moisture, especially after adding an organic admixture, the susceptibility of the composites to the development of mold fungi was tested using the swab and impression methods, which is also the novelty.

MATERIALS AND METHODS

Materials

The tests involved hemp-lime composites modified with varying amounts of acid casein. A lime-pozzolanic mixture consisting of hydrated lime CL-90s in an amount of 90% by weight and metakaolin in an amount of 10% by weight was used as a binder. Hemp shives of the Białobrzeskie variety were used as a filler (Figure 1). The mass fraction of binder to shives was assumed to be 2:1. The weight ratio of water to binder was from 1.25 to 1.40. It depended on the content of casein, which influenced the consistency of the mixture. Acid casein (Figure 2) was used as a partial substitute for the binder in amounts of 1%, 3% and 5% by weight. Casein dissolves in an alkaline environment, creating a compound with waterproof and adhesive properties. A detailed description of the prepared recipes and materials used is described in other own works, where the same ingredients were used [13, 20]. The recipes are marked as HL-0C, HL-1C, HL-3C, HL-5C, where the number indicates the percentage of casein.

The Table 1 shows the basic parameters of the composite, which are key to the analysis of the properties tested in this study, i.e. water absorption and capillary rise.

Methods

Water absorption

The mass water absorption test was performed based on the following method, already used in

Figure 1. Hemp shives used in the research

Figure 2. Acid casein used in the research

Table 1. Basic physical properties of the tested composites

Parameter	HL-0C	HL-1C	HL-3C	HL-5C
Apparent density (kg/m^3)	444.2	431.3	447.7	456.2
Total porosity (%)	79.5	80.1	79.1	78.2

own previous work [13]: Samples with dimensions of 50×60×120 mm were immersed in water and the mass increase was measured at selected time intervals. Due to the high water absorption capacity of the hemp-lime composite in the first seconds of contact with water, which is confirmed by research [15], it was decided to increase the number of readings in the initial period of immersion. The first measurement was performed after 5 s of immersion. The mass reading periods are: 5 s, 15 s, 30 s, 1 min, 15 min, 30 min, 1 h, 3 h, 12 h, 1 d, 2 d, 3 d, 5 d and 6 d. Four samples from each recipe were used for testing.

Capillary uptake

The capillary rise test was performed using TDR (Time Domain Reflectometry) probes. This method was used in own previous research on hemp-lime composites [20]. Measuring setup for TDR examination consisted of:

- sample of hemp-lime composite,
- LOM – TDR multimeter (ETest, Lublin, Poland),
- FP/mts – TDR probes (ETest, Lublin, Poland),
- PC computer as control station.

A detailed description of the principle of humidity measurement using TDR probes is described in [28]. In general, the method involves determining the apparent permittivity of porous materials by measuring the propagation time of an electromagnetic pulse along the rods of measurement probes. Samples with dimensions of 60×120×240 mm were used for the study. Side surfaces with dimensions of 60×240 mm and 120×240 mm were protected with bituminous mass to protect against water evaporation during the test and against water absorption by the side surfaces. Before test the samples were dried to constant weight at 60 °C and weighed. The use of non-invasive TDR probes in the testing of building materials, especially hard ones, is often found in the literature [29, 30], but in the case of materials with a structure such as hempcrete, the use of invasive ones is possible. Invasive FP/mts probes with 100 mm needle length were embedded at four following sample height levels 35, 85, 135, and 185 mm above the bottom edge of the sample. The sample was placed in a bathtub of water, immersing it to a depth of 10 mm (Figure 3). The water level was monitored during the study. The whole experiment lasted 5 days. Time step of the readouts was set to 15 minutes

Microbiological analysis

The susceptibility of the composites to the development of mold fungi on their surface was tested. The experiment was carried out from July to August. During this period, four recipes of composites differing in the mixture from which they were made were tested. The composites were placed at a height of approximately 1 meter and left exposed to external air for approximately 5 hours in order to contaminate them with fungal

Figure 3. General view of the sample during capillary rise test

spores found in the air. After this time, control samples were taken from the composites using the swab and impression methods to determine their number on the composite before placing them in induced conditions. The remaining composites were placed in a tightly closed box with relative humidity above 80% and temperature about 25 °C for 2 weeks and one month. After this period, swabs and prints were taken from individual batches to determine the development of fungi on the surface of the composites under the tested conditions.

The swab method involved taking a swab from the surface of 20 cm^2 of the composite using a sterile swab previously moistened in 0.85% physiological saline, which was also placed in 2 ml of 0.85% physiological saline. The swabs were then shaken for 30 sec. at a speed of 1500 RPM and a series of dilutions were performed. Samples were plated on Sabourand agar with chloramphenicol and incubated for 5–7 days at 25 °C. After this time, the grown colonies were counted and converted into CFU/cm^2 (colony-forming units/cm^2).

In the impression method, Rodac plates were used to determine the total number of yeasts and molds, which were pressed against the tested surface for approx. 10 sec. The plates were then incubated at 25 °C for 5–7 days. After this time, the grown colonies were counted and converted into cfu/cm^2. The test was performed in triplicate for each composite. During the experiment, the concentration of fungi in the outdoor and indoor air was assessed using the MAS-100 impactor. Air sampling lasted 5 minutes. The plates were then incubated at 28 °C for 7 days. After this time, the grown colonies were counted and converted into cfu/m^3.

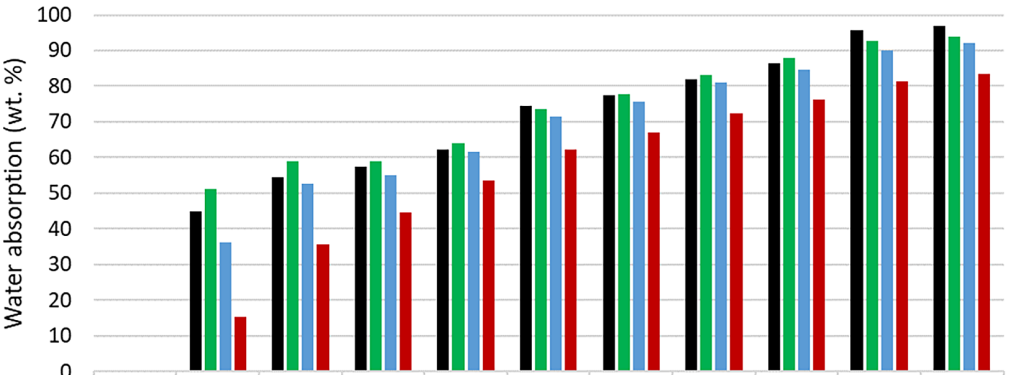
The following materials were used for microbiological analysis:

- Sabouraud Dextrose with Chloramphenicol LAB-AGAR™ – selective medium for the isolation of yeasts and molds from samples. The addition of chloramphenicol is a factor limiting the growth of most bacteria contaminating the tested material.
- Malt Extract LAB-AGAR™ – malt extract agar for the detection and enumeration of yeasts and molds used here to help identify fungal species grown on Sabouranda agar.
- Rodac ConTact Test plates for determining the total number of yeasts and molds – used to control microbiological contamination of surfaces using the contact method.
- Methylene blue – a dye used to prepare a solution for staining preparations to identify fungi under a microscope.
- Sodium chloride – preparation of a solution of sterile physiological saline used for a series of dilutions and a diluent (rinse fluid).

RESULTS AND DISCUSSION

Water absorption

The averaged values of mass absorptivity are shown in Figure 4. The maximal mass absorptivity of hemp-lime composites ranges from 83.5%



	0 s	5 s	15 s	1 min	1 h	12 h	1 d	2 d	3 d	5 d	6 d
HL-0C	0	45.0	54.5	57.4	62.2	74.3	77.6	81.9	86.5	95.5	96.7
HL-1C	0	51.2	59.0	58.9	64.1	73.6	77.7	83.1	87.9	92.6	94.0
HL-3C	0	36.2	52.7	55.2	61.6	71.5	75.7	81.0	84.7	90.1	92.0
HL-5C	0	15.3	35.6	44.6	53.6	62.2	66.9	72.3	76.3	81.4	83.5

Figure 4. Mass absorptivity results of tested composites over time

to 96.7%. The results are comparable with the data in the literature [13], where a composite with the same composition was used, but instead of casein, gum arabic was used. Similar values were also obtained in the case of lime-hemp composites with the same proportion of binder to shives [17]. The admixture of acid casein decreased water absorption. As the content of the biopolymer admixture increases, the water absorption decreases. The addition of 5% casein had the most significant impact on the results throughout the entire study period. This amount of hydrophobic substance significantly reduced the composite's ability to absorb water in the first seconds of the test (reduction by 66% after 5 s compared to HL-0C). However, the water absorption after 6 days was reduced by about 14%. As time progresses, the differences between the water absorption of HL-0C and HL-5C are becoming smaller. The hydrophobic nature of casein was also demonstrated in tests on clay mortars [31], where an admixture of 1.5% caused the mass loss of the mortar immersed in water for 30 minutes to be only 1.9%, while in the case of the reference mortar it was 79%. Typically, in the case of building materials, water absorption depends on the total porosity and increases with increasing porosity [32–34]. This tendency is also maintained in the case of HL-3C and HL-5C composites when comparing the results with the composite without casein admixture. In turn, HL-1C showed the highest porosity, however HL-0C has almost 3% higher water absorption. An analogous relationship between porosity and water absorption was observed in similar hemp-lime composites containing an admixture of gum arabic in an amount of 1% [13, 20].

Capillary uptake measurements

Figure 5 shows the increase in water volume in the sample over time based on readings from probes placed at 4 different levels. Measurements of samples HL-0C and HL-1C were terminated after 8 days and 7 days, respectively, as each probe readings showed flattened curves and no significant increase in water content was observed. However, for samples HL-3C and HL-5C, the tests were carried out further, and after the 8th day, it was noticed that in the case of HL-3C, the reading of the highest located probe was not yet stabilized, and in the HL-5C sample, probe no. 4 did not show the presence of water. Therefore, for HL-3C and HL-5C, the study was continued for up to 10 days. A longer test period was not decided due to the destructive impact of long-term water exposure to the composite. Due to the fact that in the case of the HL-1C sample the test lasted the shortest, 7 days was adopted as the period for comparing the results between the recipes.

In the case of the HL-0C composite, the rate of capillary rise of water was the highest, because already around the 40th hour of the test, water

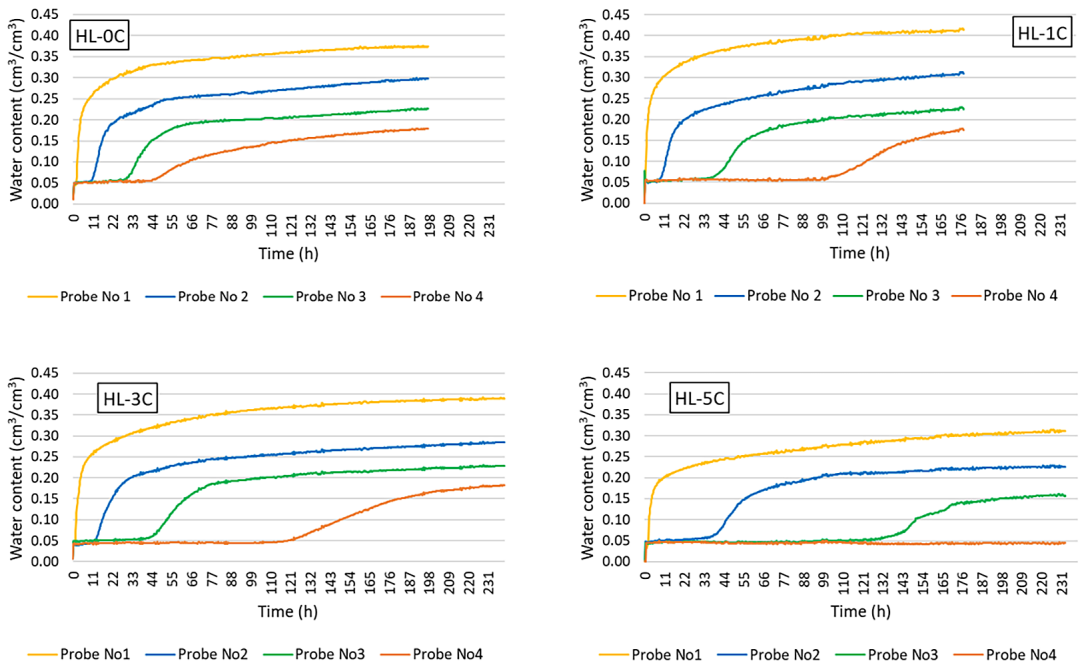


Figure 5. Capillary rise results of tested composites

was present in the area of the highest probe (No. 4), and in the area of probe No. 3, around the 30th hour of the test. As the amount of casein was increased, the rate of increase in the water column decreased because in the case of HL-1C and HL-3C, water appeared in the area of probe 4 around hour 100 and hour 120 of the study, respectively. The rate of water rising was significantly reduced in the case of the 5% casein admixture, because water appeared in the area of probe No. 3 around the 120th hour of the study, while in the area of probe No. 4 no water was observed throughout the entire test period (10 days).

The HL-1C composite, despite slower water absorption, absorbed larger amounts of water in the areas indicated by each probe compared to HL-0C. For example, the presence of water in the amount of 0.37 cm^3/cm^3 and 0.41 cm^3/cm^3 was detected in the probe No.1 area in the HL-0C and HL-1C composites, respectively, after 7 days of testing. This is the opposite situation than in the case of bulk water absorption tested by completely immersing the samples in water, where HL-1C showed lower water absorption, although on the third day of the test it showed greater water absorption. Perhaps the change was related to the possibility of damage to the structure of the samples during long-term contact with water. In the tests [13], the HL composite containing 1% of gum arabic admixture also showed greater water absorption through capillary action compared to the composite without admixtures, but in that case, the difference in the amount of accumulated water was greater. In the case of the HL-3C composite, after 7 days of testing, the amount of water on probes no. 1, 2, 3 was similar to that in the case of HL-0C. Only in the area of probe no. 4, the amount of water in the HL-3C composite was lower, but on the 9th day of testing this composite, the values equalized. This shows that 3% casein slowed down the absorption rate, but ultimately the composite was able to absorb a similar amount of water.

Based on probe measurements, accurate water content values were read using a TDR meter and volumetric water content profiles occurring at the time of the test were plotted at four levels (Figure 6). Based on the profiles, the mass of absorbed water was calculated using the formula (1) used for porous materials [17, 20].

$$m = a \cdot b \int_0^h \rho_w \theta(h) dh \qquad (1)$$

where: a and b are the sample cross-section dimensions, i.e., width and depth, (cm), h is the sample height (cm), m is the mass of absorbed water (g), θ is the moisture characteristic depending on height, i.e., volumetric moisture profile, (cm^3/cm^3), and ρ_w is the water density (g/cm^3).

Figure 7 shows the total mass of water absorbed by composites over time. Samples containing 1% and 3% casein showed similar dynamics of increase in the capillary water content as in the case of the reference sample. In the period from 24 to 192 hours of the test, the HLC composite was characterized by an average of approximately 8% lower water content compared to HL-0C. In contrast, the HL-5C samples absorbed an average of approximately 37% compared to the reference composite over this time period. Up to 48 hours of testing, the HL-1C composite was characterized by a greater ability to attract water than the composite without admixtures. This may be related to the higher porosity of the HL-1C samples. Additionally, they were characterized by poorer workability, which led to the formation of larger pores between the shives, which resulted in a higher water content, especially in the lower zones of the samples, near the water table.

Paper [16] presents the capillary rise coefficient of the lime-hemp composite after 24 hours of saturation. A composite with the same mass ratio of lime to shives was tested as in this own research. The values of this parameter were shown in the range of 2.65 and 3.37 $kg/m^2h^{1/2}$. In order to compare the capillary rise of the composites, the coefficient value was also calculated after 24 hours and the results are presented in Table 2. In the [16] tests, lower coefficient values were obtained, but these composites were characterized by a higher density of 508–627 kg/m^3.

Micorobiological analysis

The concentration of fungi in outdoor and indoor air during the experiment is presented in Table 3. The months of measurement are the turn of July and August.

Prior to exposing the samples to the external environment, both internal and external air samples were taken to assess the concentration of fungi. The concentration of fungi in the outdoor air was found to be 458 cfu/m^3, which was higher than that of the indoor air (Table 3).

The fungi that grew on the plates were identified based on the basis of macro- and microscopic

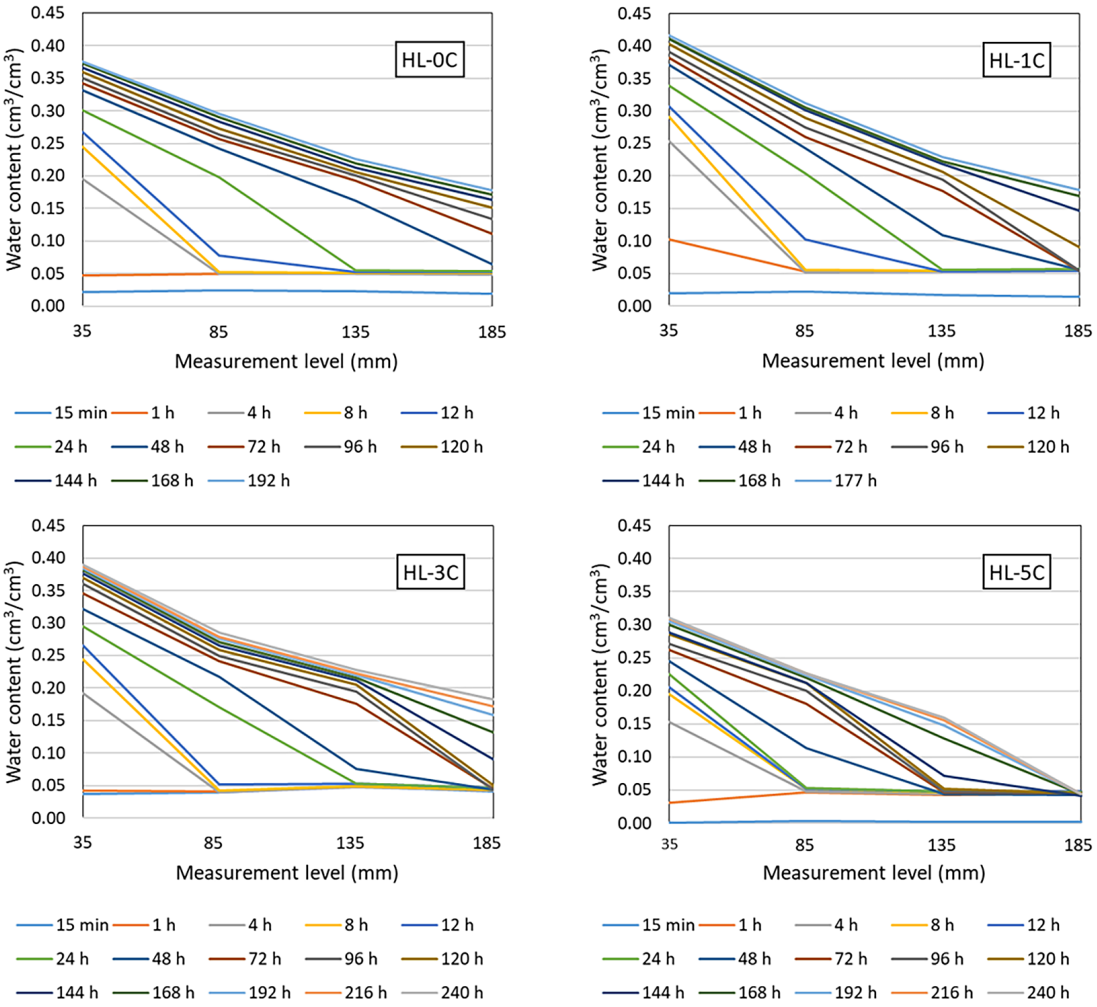


Figure 6. Moisture profiles obtained using TDR measurement in particular intervals

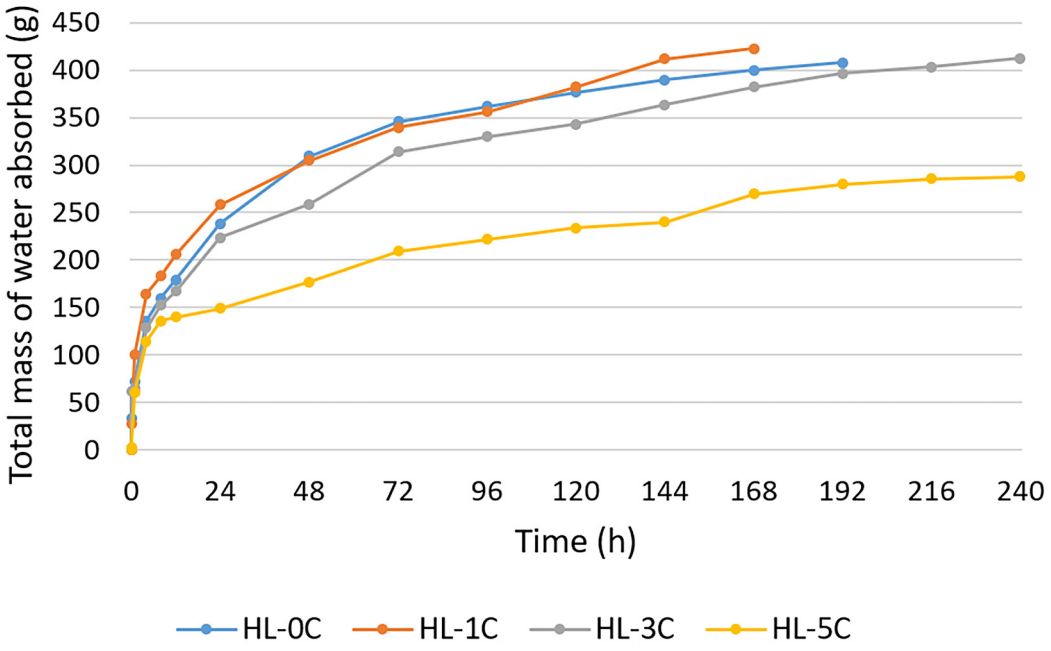


Figure 7. Total mass of water absorbed by hemp-lime composites in the function of time

Table 2. Water capillary coefficient of the tested composites after 24 hours of immersion

Parameter	HL-0C	HL-1C	HL-3C	HL-5C
Water absorption coefficient ($kg/m^2/h^{1/2}$)	6.75	7.32	6.33	4.22
Standard deviation ($kg/m^2/h^{1/2}$)	0.38	0.31	0.43	0.26

Table 3. Concentration of fungi in outdoor and indoor air during the experiment

Period of collection	Concentration of fungi in outdoor air (jtk/m^3)	Concentration of fungi in indoor air (jtk/m^3)
First measurement	458	375
Measurement 7 days later	817	542
Measurement 14 days later	1425	367

assessment using atlases and taxonomic keys. Individual species and types of fungi are listed in Table 4. The air measurements revealed the presence of the *Cladosporium* genus, whose spores are a common allergen but rarely cause opportunistic infections in humans. The second most frequently detected fungus was *Alternaria* sp., which is found in plants, soil, and indoor spaces. The types and species of fungi found in outdoor and indoor air vary depending on the day of collection. Some cultivated fungi can pose a significant threat to human health, particularly for those with weakened immune systems. *Aspergillus fumigatus*, for example, can cause acute respiratory infections known as aspergillosis

Table 5 and 6 show the numbers in individual composites and Figure 8 shows the average values of the results of testing the growth of fungi on the surface of samples. The swabs method is more accurate comparison to the printing method, especially for the composites tested, where their structure is rough. The impression method in the case of the HL-3C composite failed to download fungal spores of the genus *Chrysosporium* sp., and in the case of the HL-5C composite of the *Absida* sp. spores, which were taken using the swabs method. Individual species and types of fungi on the surface of samples both methods swab and impression shows Table 7.

Table 7 shows the identified fungi that were found on the composites studied. Similar species were found in the research [35], where indicated several species of fungi contaminating chipboard and fibreboard factories, they are *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus repens*, *Candida* spp., *Cladosporium brevicompactum*, *Geotrichum candidum*, *Mucor* spp., *Paecilomyces* spp., *Penicillium citrinum*, *Penicillium* spp., *Rhizopus nigricans*, *Rhodotorula graminis*, *Trichoderma album*, *Trichoderma viride*, *Trichothecium roseum*. Among these species, the most important in this respect are *Penicillium* and *Aspergillus* [35]. Krysińska-Traczyk et al. [36] identified mainly fungi from genera *Aspergillus* spp. including *Aspergillus fumigatus*, *Penicillium* spp., *Absidia spp*. in the air of factories making furniture from different materials: beech wood, fibreboards chipboards. Korpacz and Fojnowski [37] studied colonization of wood chips by fungi. The most common were fungi of the genus *Penicillium* spp. Also appearing was the *Alternaria* sp. genus.

Table 4. Species and types of fungi occurring in outdoor and indoor air

Period of collection	Fungi occurring in outdoor air	Fungi occurring in indoor air
First measurement	*Alternaria* sp., *Cladosporium* sp. *Rhizopus* sp., *Aspergillus* sp., *Aspergillus clavatus*	*Alternaria* sp., *Cladosporium* sp., *Trichoderma* sp., *Aspergillus flavus,*
Measurement 7 days later	Predominance: *Alternaria* sp. and *Cladosporium* sp. Present: *Fusarium* sp.	*Penicillium* sp., *Cladosporium sp., Scopulariopsis* sp. *Acremonium* sp., *Aspergillus fumigatus, Aspergillus flavus*
Measurement 14 days later	Predominance: *Cladosporium* sp. Present: *Alternaria* sp. and *Fusarium* sp.	Predominance: T*richoderma* sp. Obecne: *Cladosporium* sp., *Penicillium* sp.

Table 5. Results of testing the growth of fungi on the surface of composites – swab method

Control samples							
HL-0C	jtk/cm^2	HL-1C	jtk/cm^2	HL-3C	jtk/cm^2	HL-5C	jtk/cm^2
HL-0C_1	15	HL-1C_1	11	HL-3C_1	8	HL-5C_1	6
HL-0C_2	5	HL-1C_2	6	HL-3C_2	0	HL-5C_2	5
HL-0C_3	8	HL-1C_3	6	HL-3C_3	5	HL-5C_3	8
Average	9	Average	8	Average	4	Average	6
After two weeks							
HL-0C	jtk/cm^2	HL-1C	jtk/cm^2	HL-3C	jtk/cm^2	HL-5C	jtk/cm^2
HL-0C_1	0	HL-1C_1	0	HL-3C_1	6	HL-5C_1	6
HL-0C_2	0	HL-1C_2	0	HL-3C_2	0	HL-5C_2	0
HL-0C_3	0	HL-1C_3	8	HL-3C_3	126	HL-5C_3	8
Average	0	Average	3	Average	45	Average	5
After one month							
HL-0C	jtk/cm^2	HL-1C	jtk/cm^2	HL-3C	jtk/cm^2	HL-5C	jtk/cm^2
HL-0C_1	22	HL-1C_1	17	HL-3C_1	102	HL-5C_1	0
HL-0C_2	0	HL-1C_2	8	HL-3C_2	5	HL-5C_2	0
HL-0C_3	58	HL-1C_3	8	HL-3C_3	17	HL-5C_3	2,6 x 10^2
Average	27	Average	11	Average	41	Average	87

Table 6. Results of testing the growth of fungi on the surface of composites – impression method

Control samples							
HL-0C	jtk/cm^2	HL-1C	jtk/cm^2	HL-3C	jtk/cm^2	HL-5C	jtk/cm^2
HL-0C_1	2.9	HL-1C_1	3	HL-3C_1	5.5	HL-5C_1	4.8
HL-0C_2	2.8	HL-1C_2	2.3	HL-3C_2	6.2	HL-5C_2	6.3
HL-0C_3	2.2	HL-1C_3	3.4	HL-3C_3	5.9	HL-5C_3	4.1
Average	2.6	Average	2.9	Average	5.8	Average	5.1
After two weeks							
HL-0C	jtk/cm^2	HL-1C	jtk/cm^2	HL-3C	jtk/cm^2	HL-5C	jtk/cm^2
HL-0C_1	0.3	HL-1C_1	0.7	HL-3C_1	0	HL-5C_1	0.2
HL-0C_2	0.3	HL-1C_2	0.4	HL-3C_2	0	HL-5C_2	0.2
HL-0C_3	0.2	HL-1C_3	0	HL-3C_3	0	HL-5C_3	0.1
Average	0.3	Average	0.4	Average	0	Average	0.2
After one month							
HL-0C	jtk/cm^2	HL-1C	jtk/cm^2	HL-3C	jtk/cm^2	HL-5C	jtk/cm^2
HL-0C_1	0	HL-1C_1	1.1	HL-3C_1	0.4	HL-5C_1	0.6
HL-0C_2	0	HL-1C_2	0.4	HL-3C_2	0.5	HL-5C_2	0.4
HL-0C_3	0						
Average	0	Average	0.7	Average	0.4	Average	0.5

For composites tested using the impression method, a decrease in fungal growth and presence can be observed after only two weeks. However, the swab method for HL-5C composites shows a similar number of fungi on the surface after two weeks and an increase in their number after a month compared to the control samples. This is due to the presence of spores from the *Absidia* genus, which are commonly found in nature. However, in the case of the HL-3C composite, higher values were observed after 2 weeks and a month in comparison to the control samples. This is attributed to the presence of spores from the genus *Chrysosporium* sp. The differences in the number of fungi are due to the composition of the composite, which

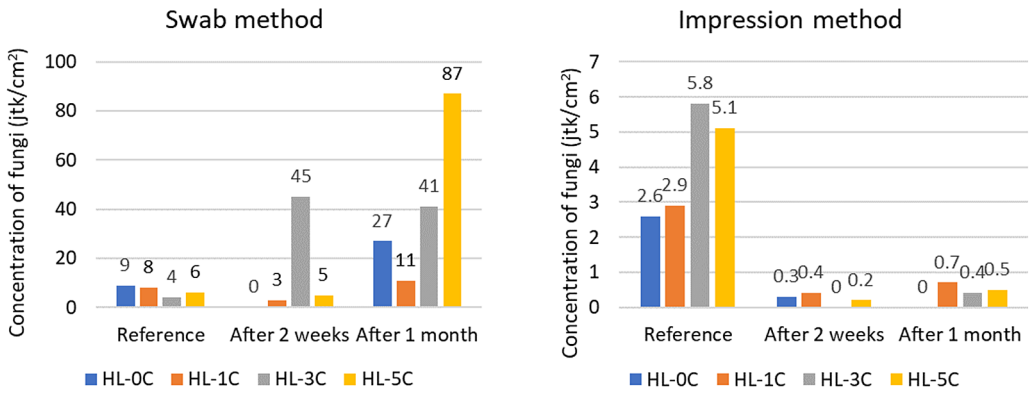


Figure 8. Average values of the results of testing the growth of fungi on the surface of samples

Table 7. Fungi identified from the composite surface using swab and impression methods

Composite symbol	Swab method	Impression method
	Control samples	
HL-0C	Predominance: *Cladosporium* sp., *Cladosporium herbarum, Fusarium* sp., *Cladosporium* sp., drożdże, *Aureobasidium* sp., *Mucor* sp. *Absidia* sp.,	*Alternaria* sp., *Cladosporium* sp., *Fusarium* sp., *Rhizomucor* sp., *Verticullum* sp. *Absidia* sp., *Geotrichum* sp.,
HL-1C	*Cladosporium* sp., *Alternaria* sp. *Rhizomucor* sp. *Geotrichum* sp., *Culvularia* sp. *Acremonium* sp., *Cladosporium herbarum*	Predominance: *Aspergillus versicolor* Present: *Cladosporium* sp., *Alternaria* sp. *Aspergillus niger, Aspergillus fumigatus, Mucor* sp. *Fusarium* sp.,
HL-3C	Predominance: *Cladosporium* sp., *Alternaria* sp. *Acremonium* sp., *Mucor* sp., *Rhodotorula mucilaginosa, Trichoderma* sp.	Predominance: *Aspergillus versicolor* Present: *Cladosporium* sp., *Alternaria* sp., *Acremonium* sp., *Scopulariopsis brevicaulis*,
HL-5C	*Cladosporium* sp., *Alternaria* sp., *Geotrichum* sp., *Penicillium* sp., *Fusarium* sp., *Acremonium* sp.,	Predominance: *Aspergillus versicolor* Present: *Cladosporium* sp., *Alternaria* sp., *Geotrichum* sp.,
After two weeks		
HL-0C		Predominance: *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., *Fusarium* sp., *Rhizomucor* sp., *Chrysosporium* sp.,
HL-1C	*Cladosporium* sp., *Cladosporium herbarum Acremonium* sp., *Penicillium* sp.,	*Chrysosporium* sp., *Ulocladium* sp., *Acremonium* sp., *Penicillium* sp., *Fusarium* sp. *Cladosporium* sp
HL-3C	*Cladosporium* sp., *Alternaria* sp. *Scopulariopsis brevicaulis Chrysosporium* sp., *Ulocladium* sp. *Penicillium* sp.,	
HL-5C	*Penicillium* sp., *Cladosporium* sp., *Acremonium* sp., *Alternaria* sp. *Ulocladium* sp. *Aspergillus versicolor Chaetomium* sp., *Fusarium* sp.	*Verticullum* sp., *Ulocladium* sp., *Absidia* sp., *Culvularia* sp., *Aspergillus fumigatus*
After one month		
HL-0C	Predominance: *Cladosporium* sp., *Present*: *Absidia* sp., *Aspergillus niger, Rhizomucor* sp. *Ulocladium* sp.	
HL-1C	Predominance: *Aspergillus flavus,* Present: *Aspergillus versicolor, Cladosporium* sp., *Cryptococcus* sp., *Penicilium* sp., *Ulocladium sp., Alternaria* sp.	Predominance *Penicillium* sp., Present: *Aspergillus niger, Alternaria* sp.
HL-3C	Predominance: *Chrysosporium* sp., Present *Scopulariopsis brevicaulis*, *Aspergillus versicolor, Aspergillus flavus, Aspergillus fumigatus*	*Scopulariopsis brevicaulis*, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium* sp., *Culvularia* sp.
HL-5C	Predominance *Absida* sp., Present *Cladosporium* sp.	*Ulocladium* sp., *Aspergillus versicolor, Aspergillus fumigatus, Aspergillus niger, Penicilium* sp.

allowed the spores to survive and grow. Photos (Figures 9–14) depict the development of fungi on the impression plates, as well as from the swab method in selected samples.

Figures 9 and 10 show the results from the swab method after two weeks and one month of incubation of the composite in two replicates, which show the growth of *Chrysosporium* sp. fungi. No growth of *Chrysosporium* sp. fungus was noted in the control samples.

Figure 11 shows the growth of a fungus of the genus *Absidia* sp. after one month of incubation

Figure 9. Swab method: composite HL-3C – after 2 weeks, the predominance of the genus *Chrysosporium* sp.

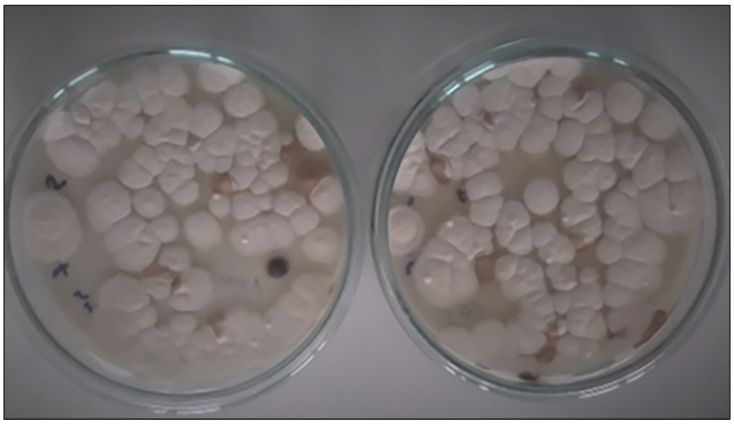


Figure 10. Swab method: composite HL-3C – after 1 month, the predominance of the genus *Chrysosporium* sp.

Figure 11. Swab method: HL-5C composite – after 1 month, the predominance of the genus *Absidia* sp.

obtained using the swab method in duplicate. As in the case of HL-3C, no growth of this genus was detected with respect to the control samples.

Sand et al. in work they think an important factor in the development of moulds on building materials is their biodeterioration activity, causing damage of the construction environment [38, 39]. Many microorganisms are capable of metabolizing organic compounds via fermentation. As a result, other organic compounds are formed, which, in many instances are organic solvents. Organic acids like acetic, formic, or butyric acid as well as alcohols (ethanol, propanol, butanol, etc.) and ketones are noteworthy. These solvents may react with materials of natural and/or synthetic origin, causing swelling, total or partial dissolution and, finally, deterioration. Additionally, the microorganisms growing on and/or in mineral materials often excrete exopolymers Exopolymers contain ionic groups, which cause them to function like ion-exchangers. Together with metabolic end-products like acids (and the derived salts), an increased water content of porous materials

results. The consequences have been described above. Biofilm and its exopolymers aggravate this problem by clogging the pores of materials reducing evaporation of water [38].

The impression method showed that the number of fungal spores on the composites tested was high in the control samples (Figure 12). However, after two weeks (Figure 13) and one month of storage in induced conditions (Figures 14), on the composites tested with the addition of casein, their number decreased significantly. Comparing the fungi grown on the composites after two weeks and one month, it can be observed that fungi of the genus *Penicillium* sp. and *Alternaria* sp. are mainly present.

Some species of the genus *Penicillium* and *Cladosporium* are used to grow on wood even at low temperatures, approximately 5 °C The world is supervisory, which can influence fungal spores and their rapid growth, as well as the synthesis of some component parts [40, 41]. The growth of microorganisms on these materials was supported not only by their chemical composition, but also by their geometric structure, e.g. surface irregularities. Maintaining an appropriate level of moisture on the surface of these materials is only necessary for the development of fungi in the initial stages of growth. Once the mould has formed a mycelium, the moisture is retained in its structure, allowing most hydrolytic enzymes to be active [40]. Rojas et al. [42] showed in their work that the moulds *Penicillium*, *Aspergillus*, *Stachybotrys*, *Cladosporium* and *Alternaria*, which produce the enzymes cellulase and amylase, can cause the biodegradation of various types of paper used for technical purposes.

The starch and casein-based glues used to adhere wallpaper and cardboard to plaster are also degraded by extracellular enzymes and can provide a carbon source for moulds [43]. De La Torre and Gomez-Alarcon [44] showed that moulds grew much slower in the medium containing mortar, reaching 60% less mycelial biomass and the amount of mycelial components chitin, glucan, ergosterol decreased by 13–41%. This is evidence of the presence in the mortar of a factor that inhibits

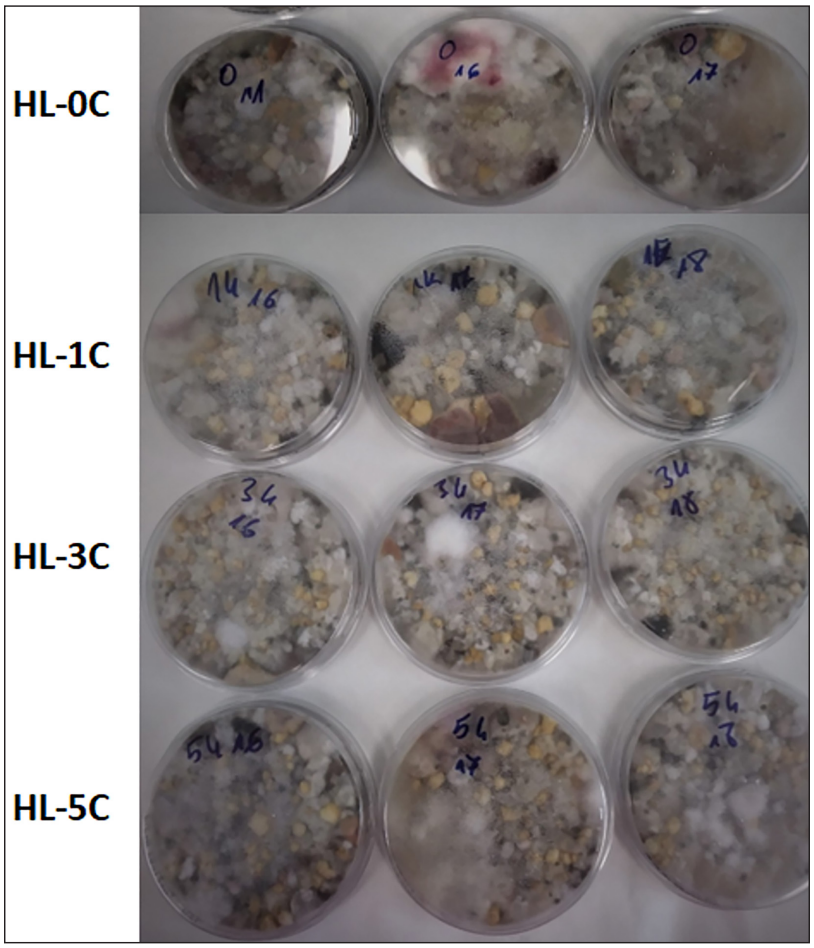


Figure 12. Impression method: control samples

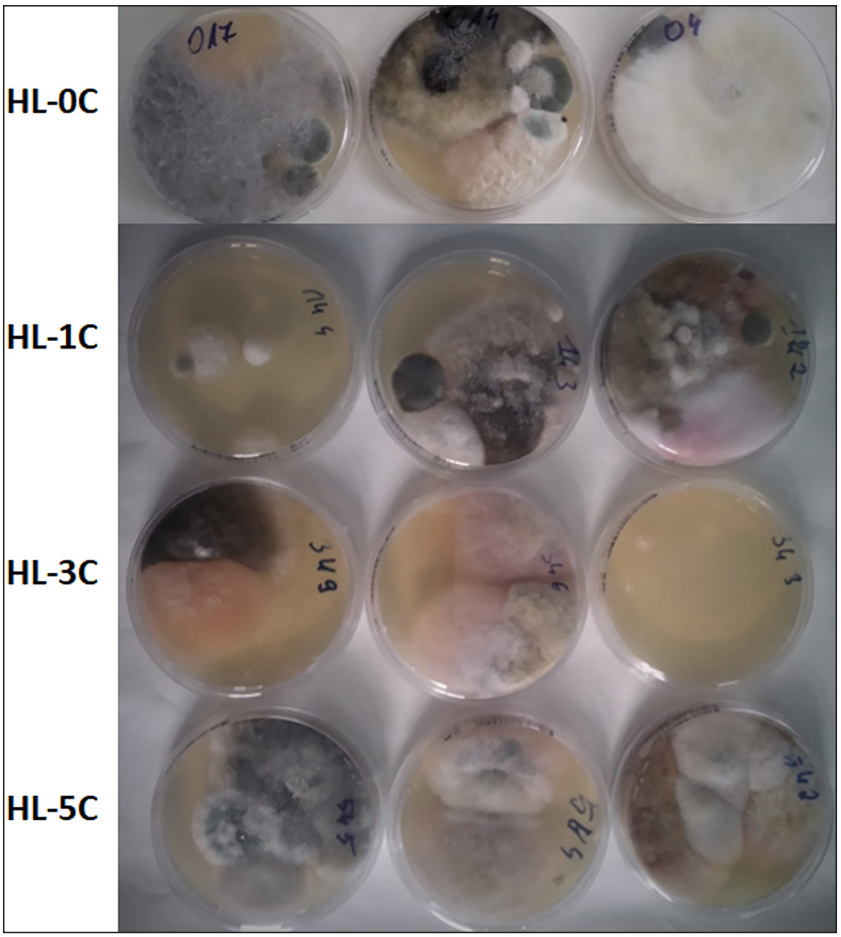


Figure 13. Impression method: after two weeks

mould growth, such as high pH, but also the presence of chemical compounds that inhibit the synthesis of cellular components ($CaSO_4$, oxides of Ca, Al, Si, Fe, Mg, K, Na, S, possibly biocides). The presence of inorganic compounds from the mortar in the medium also significantly inhibited the production of extracellular enzymes. This may have been due to the initial pH of the medium, particularly mortar (pH = 10.2), which reduced the activity of most enzymes. The high pH of the mortar probably directed the mould metabolism towards the production of organic acids. The organic acids produced by *Penicillium, Aspergillus* and *Trichoderma* moulds are highly corrosive [44, 45]. Gutarowska [40] presented differences in the production of enzymes and organic acids by moulds when growing on media with the addition of building materials of organic and inorganic origin indicate two mechanisms of activity of moulds in the biodeterioration of building materials. Having an available carbon source and optimum pH, on organic materials moulds produce principally mycelium and extracellular enzymes, while on inorganic materials, where mycelium growth is impeded, they increase production of organic acids. Organic acids produced in oligotrophic conditions by moulds may be the reason for microbial corrosion on inorganic building materials [40].

CONCLUSIONS

The research concerns significant problems related to composites based on organic ingredients of plant and animal origin. The ability to absorb water and susceptibility to the development of microorganisms were tested. Based on the obtained research results, the following conclusions can be formulated:

- studies on water saturation of the composite have shown that acid casein combined with a basic lime binder has a hydrophobic effect, limiting the water absorption capacity of the hemp-lime composite;
- after 6 days of saturation, mass water absorption of the composite was from 83.5 to 96.7%. The addition of casein reduced the values of this parameter. As the biopolymer content

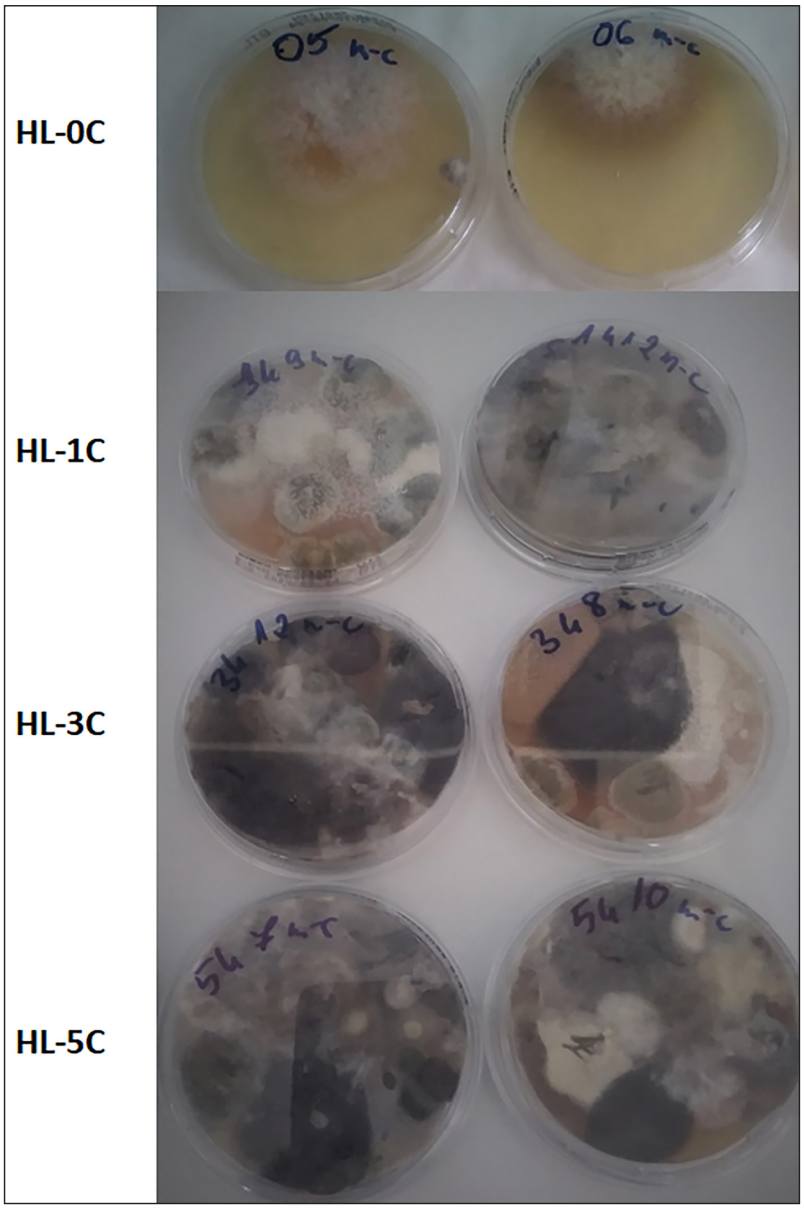


Figure 14. Impression method: after one month

increased, the water absorption decreased. The composite with an admixture of 5% was characterized by a 14% lower value of this parameter compared to the reference composite;

- the addition of 5% casein significantly reduced the amount of water drawn up by capillary action, the rate of rising and the height to which the water was drawn up. After 24 hours of testing, the capillary rise coefficients were 37% lower than in the case of the composite without admixtures;
- the structure and composition from which the composite is made affects the survival and development of fungi. The most common fungus in both control samples and after 2 weeks and a month of incubation was the type of *Cladosporium* sp. and *Alternaria* sp., which shows that the composition was created allowed to survive. The HL-5C composite has also caused an increase in fungus of the genus *Absida* sp.;
- under the assumed research conditions, the fungi appeared only on the surface of the samples, and the visulars in many cases were not noticeable. Did not cause damage to the structure. It can therefore be concluded that despite the presence of shives and casein, the alkaline reaction of lime provides protection with composites.

After demonstrating the positive effect of the admixture of casein, especially in the amount of 5%, on reducing the ability of the hemp-lime

composite to absorb water, as well as its neutral effect on the level of mold development, it is worth examining other properties. The authors conduct research on the influence of casein on the mechanical and thermal properties of the composite, as well as those related to water vapor transport.

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