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A Study on Sensitivity of Soil-Based Building Mixtures to Biodeterioration by Fungi: Towards Sustainable Earth Structures

Amer Al-Jokhadar [1,*], Yasmine Soudi [1], Suzanne Abdelmalek [2], Sarah R. Badran [1] and Yasser Abuhashem [1]

[1] Department of Architecture, Faculty of Architecture and Design, University of Petra, Amman 11196, Jordan; ysoudi@uop.edu.jo (Y.S.); sarahrbadran@gmail.com (S.R.B.); dr-abuhashem@gmx.de (Y.A.)
[2] Department of Pharmacology and Biomedical Sciences, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman 11196, Jordan; sabdelmalek@uop.edu.jo
* Correspondence: amer.aljokhadar@uop.edu.jo

Abstract: Earth structures have a significant sustainable impact on regulating indoor environmental qualities. Yet, using soil materials can lead to fungal growth, impacting occupant health and structural stability. This study investigates the susceptibility of earth-based construction materials with cement, limestone, and acrylic-based additives to fungal growth. Laboratory tests were conducted on mixtures under conditions found in inhabited buildings in hot–arid regions. The proposed methodology was based on a 7-week artificial incubation of fungi obtained from moldy walls through regulating the room temperature to fall between 18 °C and 19 °C and a controlled humidity level of around 45%. These conditions were adopted according to the readings monitored in typical buildings in the study area. The results showed that fungal growth was evident on the surface of mixtures, including higher percentages of soil and lower percentages of additives. Mixtures comprising 50% soil, 15% acrylic-based additive, 15% quicklime, and 20% cement supported the least fungal growth, presenting the best choice as a sustainable, efficient replacement. Visual observation followed by microscopic examination ensured the results. Furthermore, results of an environmental post-occupancy evaluation of a constructed rammed earth building using the optimized mixture showed no signs of fungal proliferation on the inner walls afterward.

Keywords: fungal growth; mold; soil; humidity; rammed earth; green solution; sustainability

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1. Introduction

The attention garnered by utilizing local resources in constructing sustainable structures, such as soil, stems from its significant environmental impact. This noteworthy method not only contributes to the enhancement of air quality but also aids in regulating temperature and humidity, ultimately ensuring a pleasant living environment for the inhabitants of the structures [1–4]. Furthermore, it diminishes the dependence on energy resources for heating and cooling [5–7].

However, such a building system can enhance fungal growth. Fungi are abundant in nature. They can grow on almost all natural and manmade materials. Depending on the original location, an ambient temperature between cold and moderate levels is the optimum requirement for most fungi [8]. Additionally, a correlation was established between the moisture content during the construction of earth walls and its possible influence on mold development [9,10]. Fungal growth potentially impacts the health of occupants and the structural stability of the buildings [11]. This is because of the capacity of earth materials to absorb water, and hence, the moisture content within materials and the humidity levels on their surface determine the rate at which fungi develop [12] and subsequently impact the structure's durability [13–16]. Laborel-Préneron et al. (2018) studied earth-based materials enhanced with plant aggregates such as straw [17]. These enhancements improved insulation and helped prevent shrinkage cracks; however, they

also observed fungal growth under specific humidity and temperature conditions after four weeks. Mensah-Attipoe et al. (2015) focused their research on the proliferation of environmentally friendly construction materials, shedding light on how organic matter facilitates the growth of fungi [18].

To mitigate the effects of fungal growth on the durability of earth constructions, it is possible to modify a combination of mixtures, reduce moisture, and ensure the maintenance of the building [19]. Lime, fly ash, cement, polymers, and red clay binders with epoxy emulsion have been used as additives to improve the properties of soils against water erosion [20–25]. Moreover, reducing clay content in mixtures has proven effective [26,27]. Narloch and Woyciechowski (2020) conducted a study using the Standard NZS 4298 by incorporating a cement dosage of 6% into the mixture [28]. Their findings indicated that there were no signs of surface degradation. Yet, they emphasized that protective measures are necessary for long-term resilience. Moreover, it was found that adding cement to earth mixtures can improve resistance to water vapor, although it is still not as effective as concrete. Another method involves changing the mixture composition by incorporating admixtures, like silicone water additives, which help protect against the impact of weathering. Additionally, surface treatments can address moisture-related issues [29].

Since earthen building materials are one of the green solutions for contemporary constructions, and fungal growth can lead to health risks for occupants, questions are raised about the optimized soil mixture to be used in hot–arid regions that reduces fungal development. This study investigates susceptibility to fungal growth on the surface of earth constructions. Additionally, it aims to explore the impact of adding cement, limestone, and a fast acrylic-based bond to a mixture for moisture resistance. Laboratory tests were conducted on mixtures under conditions found in inhabited buildings in hot–arid regions, where fungal growth is expected. The study's findings will highlight the optimized mixture of a stabilized earth material that prevents fungal growth and maintains acceptable indoor air quality. The selected mixture was used to construct a prototype rammed earth building in the study area to examine the efficiency of such material in preventing mold growth.

2. Materials and Methods

The experiment in this study was performed in Amman, Jordan, a city recognized for its hot and arid summer climate, juxtaposed with its cold and damp winter climate. In this regard, the mean percentage of outdoor relative humidity is 48% [30]. Moreover, the mean maximum outdoor air temperature for 30 years (1989 to 2018) ranged between 32.8 °C and 12.6 °C, with an average of 23.9 °C annually, while the mean minimum outdoor air temperature ranges between 20.8 °C and 3.6 °C, with an average of 12.7 °C, annually [31].

Regarding indoor environmental conditions, profile readings of previous studies implemented in the same study area show that the mean indoor air temperature monitored in typical buildings in Amman ranges between 17.2 °C and 19.4 °C in cold months (November to February), while relative humidity levels fall between 42% and 48% in the same months [32,33]. Research examining the indoor environmental quality (IEQ) of typical classrooms in Amman in winter (between December and January) showed that the relative humidity measured between 43.5% and 55.2%, with a mean of 47.6%. In the same surveyed rooms, the indoor air temperature varied between 17.3 °C and 21.4 °C, with a mean of 19.35 °C [32]. In another study aimed to assess the IEQ of schools in Amman, results showed that air temperature ranged between 16 °C and 18.3 °C in February, and between 21.1 °C and 22.9 °C in July [33]. Relative humidity measurements ranged between 42% and 47% in winter, while it was recorded between 40.5% and 47.9% in summer [33].

Different techniques and laboratory tests could be used to investigate fungal growth at the surface of building materials. The first step includes preparing mixture samples and then decontaminating them to remove mold by exposing materials to heat [34] or treating them with gamma rays, which is more expensive [35].

The second step is to collect fungi from moldy walls and inoculate them artificially at the surface of the mixture samples using a cotton swab [36]. This process is faster than natural inoculation and allows an easier quantitative comparison with the initial state [37].

The third step is to incubate samples in a closed chamber to maintain controlled temperature and relative humidity, similar to regular room conditions. The average time of the incubation process is six weeks [37].

Finally, periodic macroscopic and microscopic observations are used to monitor fungal growth. Depending on the inoculation method, different scales that classify fungal growth intensity could be used. One of these scales was established by Johansson et al. in 2012 [38]. It includes five values, starting from 0 to 4. The lowest value means no fungal growth, while the highest value indicates growth all over the surface. Health risks are associated with growth values of 2 and above.

The procedure, explained in the subsequent paragraphs, is focused on thoroughly examining the laboratory tests employed to observe fungal growth.

2.1. Preparation of Samples and Decontamination

A total of six types of mixtures, with three replicates of each type, were prepared, as shown in Table 1. The six mixtures are as follows: three types of different soil mixtures with additives, a control sample that includes soil without any additives, a concrete mixture without any treatment, and a concrete mixture that is painted with emulsion paint. Soil obtained from a depth of two meters was used to prepare mixtures to avoid silt and high concentrations of organic material. The reason for choosing these types of soil mixtures is to select the optimum combination that can be used for contemporary earth constructions and compare soil with ordinary Portland cement commonly used in the study area. It is essential to mention, though, that straws or plants, which were found to be a major cause of fungal growth, were not used in the mixtures [35,39,40].

Table 1. Description of types of samples.

Type Code	Composition of Samples
(1A)	Soil (70%) Acrylic-based additive (15%) Quicklime (15%)
(2B)	Soil (65%) Acrylic-based additive (15%) Ordinary Portland cement (20%)
(3C)	Soil (50%) Acrylic-based additive (15%) Quicklime (15%) Ordinary Portland cement (20%)
(4D) Control sample	Soil (100%) with no additions
(5E)	Concrete mixture (100%)
(6F)	Concrete mixture (100%), finished with emulsion paint

At an earlier phase of the current study, a laboratory structural investigation was performed to verify the strength of soil mixtures. The results showed that the soil mixture denoted as type (1A) accomplished a compressive strength of 2.06 MPa after 28 days while being subjected to a water content of 37%. The sample type 2B yielded a compressive strength valued at 4.18 MPa and a water content of 33%. Surprisingly, the sample labeled as type 3C manifested the highest compressive strength of 4.39 MPa while being exposed to a water content of 42% [41].

All samples underwent decontamination, exposing them to exceptionally elevated temperatures in an oven that reached up to a scorching 150 °C for 24 h. The fundamental

aim of this process was to successfully eliminate any probable existence of mold, thereby ensuring the integrity and purity of the samples under investigation.

2.2. Inoculation

Fungal specimens were obtained from mold-infested walls of residential dwellings in Jordan to simulate natural conditions (Figure 1). Wet sterile cotton swabs were used to swab moldy walls. These swabs were then cultured on Sabouraud Dextrose Agar (SDA). These cultures were used to inoculate the samples in question. The inoculation was carried out at room temperature, set at 24 °C, to be within the acceptable comfort range, between 19.4 °C and 27.7 °C, as defined in the ANSI/ASHRAE 55 (2017) guidelines [42].

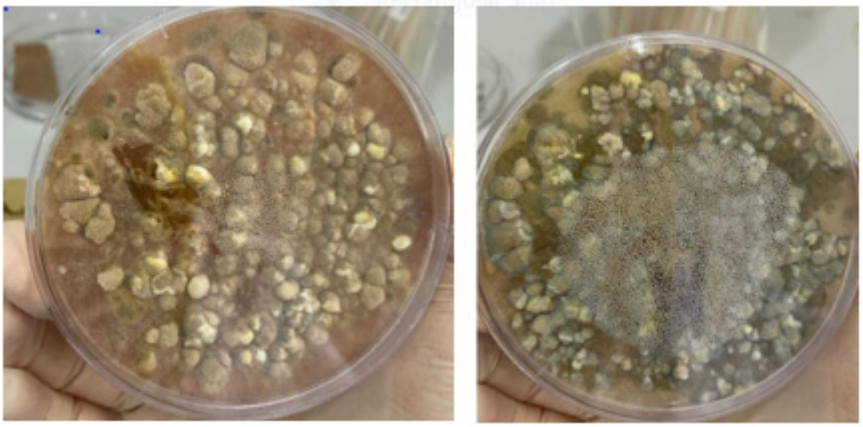


Figure 1. Fungal growth was obtained from mold-infested walls of residential dwellings in Jordan.

Sterile and immaculate Petri dishes were marked with the precise date and their unique material code, which can be observed in Figure 2:

- Label (1A): 80% soil, 5% acrylic-based additive, and 15% quicklime.
- Label (2B): 65% soil, 15% acrylic-based additive, and 15% cement.
- Label (3C): 50% soil, 15% acrylic-based additive, 15% quicklime, and 20% cement.
- Label (4D): 100% soil (control sample).
- Label (5E): 100% concrete mixture.
- Label (6F): 100% concrete mixture, painted with emulsion.

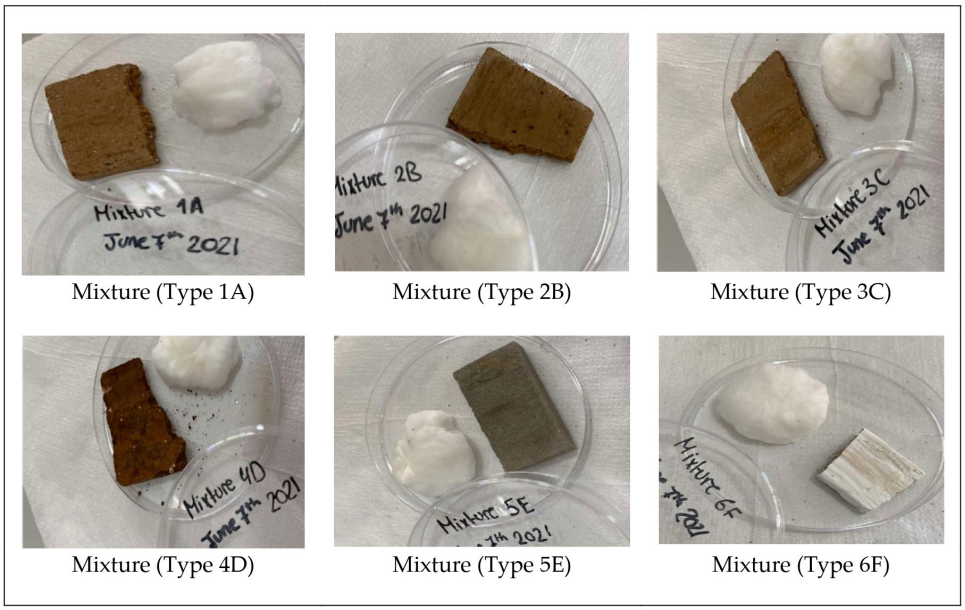


Figure 2. Mixtures of earth construction samples were used in the study.

To execute the procedure, it was necessary to implement a consistent and unvarying relative humidity factor, which was maintained at approximately 45%. This value was carefully chosen to ensure that it simulated the indoor environmental conditions of typical buildings in the study area.

Standardization of Humidity Level

The test commenced on the 10th of May, with the primary focus being observing petri dishes containing solely the material samples following inoculation with fungi. However, the humidity levels were significantly low, measuring approximately 25%. To reach the required level of humidity, which is 45%, a quantity of cotton wool was thoroughly saturated with (2 to 4) water sprays. Subsequently, this wet cotton wool was placed alongside a sample in a petri dish (Figure 3). A susceptible wireless transmitting sensor was incorporated within the same petri dish to measure relative humidity (RH) levels and indoor air temperature (Figure 4). Specifications of the used instrument are shown in Table 2. A 45% RH threshold was successfully achieved on the 7th of June. To maintain the desired humidity level of approximately 45%, the samples were kept under observation, and the cotton wool was consistently re-sprayed each time the samples were assessed.

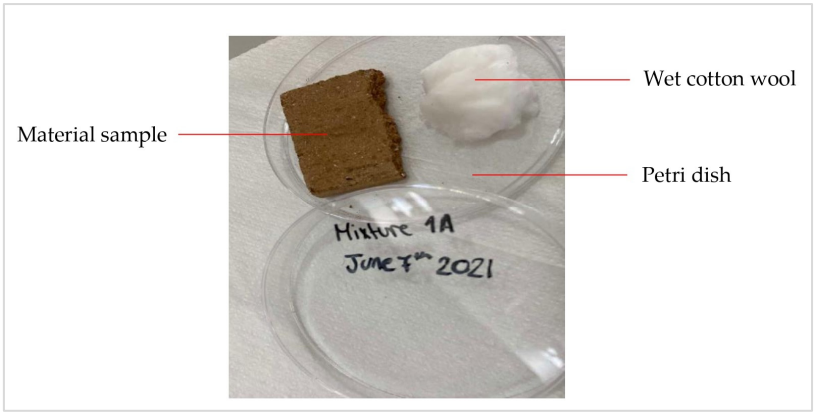


Figure 3. Incubation setup.

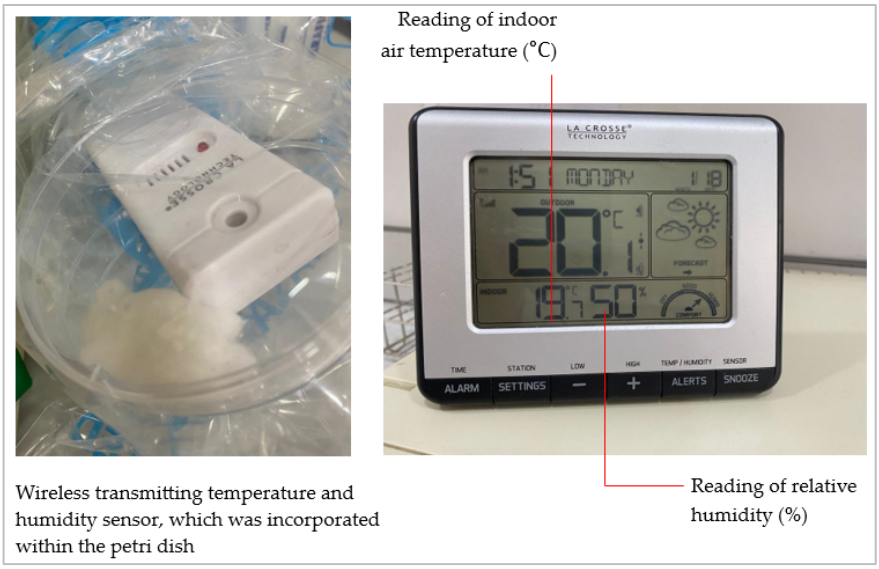


Figure 4. Humidity- and temperature-measuring devices were used in the experiments.

Table 2. Specifications of the instrument used to measure relative humidity and air temperature.

Investigated Variable	Specifications of the Measuring Tool
Indoor air temperature	Temperature sensor Indoor temperature range: 0 to 50 °C Accuracy: ±0.6 °C Resolution: 0.1 °C Update interval: every 31 s
Relative humidity	RH sensor Indoor humidity range: 10% to 99% Accuracy: ±3% RH Resolution: 0.1% RH Update interval: every 31 s

2.3. Incubation

Samples were incubated for seven weeks, from the 7th of June to the 25th of July. Throughout this time, meticulous measures were implemented to maintain optimal conditions for the experiment, specifically by regulating the room temperature to fall within the range of 18 °C and 19 °C. Furthermore, humidity levels were carefully controlled to fluctuate between 45% and 50%. These specific parameters were selected due to their suitability in fostering the growth of fungi within the study area and to ensure the simulation of indoor environmental conditions of typical buildings in Jordan.

The temperature and humidity conditions of the incubation process during the experiment are presented in Table 3. The experiment, which spanned 49 days, was subject to an unwavering commitment to precision and accuracy, ensuring that the data obtained would be reliable and robust.

Table 3. Temperature and humidity conditions of the incubation process.

	Week #1	**Week #2**	**Week #3**	**Week #4**	**Week #5**	**Week #6**	**Week #7**
	From 7/6 to 13/6	**From 14/6 to 20/6**	**From 21/6 to 27/6**	**From 28/6 to 4/7**	**From 5/7 to 11/7**	**From 12/7 to 18/7**	**From 19/7 to 25/7**
Average Relative Humidity	45%	49%	46%	45%	43%	43%	40%
Average Room Temperature	18 °C	18.9 °C	19 °C	18.3 °C	18.7 °C	19.1 °C	18.4 °C

2.4. Macroscopic and Microscopic Examination

The samples were examined macroscopically during the 7th week for visual signs of mycelial growth or spores. After a long examination period, substantial results were recorded to facilitate comparison.

2.5. Microscopic Observation

Samples were swabbed properly from all sides of the specimen with a wet swab, and the swab was then mixed in a drop of the lacto phenol blue stain and placed on a clean and dry microscopic slide. The smear was then covered with a cover slip and observed under a bright field microscope at 400× magnification.

2.6. Environmental Post-Occupancy Evaluation for the Experimental Rammed Earth Building

An experimental rammed earth building was constructed in the study area using the optimized mixture with the least fungal growth. An environmental post-occupancy study was conducted during the winter season, between December 2022 and March 2023, to assess fungal growth. The indoor air temperature and relative humidity levels inside the building were measured using a susceptible instrument (Table 4). The tool was calibrated according to the manufacturer's instructions and ANSI/ASHRAE 55 specifications [42] and was firmly positioned within the confines of the room to measure and document the readings accurately.

Table 4. Specifications of the instrument used to measure indoor environmental conditions.

Investigated Variable	Specifications of the Measuring Tool
Indoor air temperature	Temperature sensor Measuring range: −10~60 °C Accuracy: ±0.6 °C Resolution: 0.1 °C Time interval: 5 s
Relative humidity	RH sensor Measuring range: 0.1~99.9% Accuracy: ±3% RH (at 25 °C, 10~90% RH) Resolution: 0.1% RH Time interval: 5 s

3. Results

The experiment mentioned above was conducted to ascertain if specific combinations of construction materials are susceptible to fungus development. After infecting the samples with fungi, the results obtained are as follows.

During the second and third weeks, specifically from the 16th to the 24th of June, morphological alterations were observed on the surface of the samples labeled (1A), (2B), and (4D), as shown in Figure 5. It should be noted that fungal growth was observed in the wet cotton wool in petri dishes on the 16th of June.

On the 24th of June, when the relative humidity stood at 45%, and the room temperature was recorded as 18.3 °C, fungal growth at the surface of samples was rated based on the scale established by Johansson et al. in 2012 [38]. The samples labeled (1A) and (4D) received a rating of 2 due to minimal growth. On the other hand, the sample (2B) received a rating of 1, signifying the commencement of growth. The remaining three samples, labeled (3C), (5E), and (6F), were rated 0, indicating the absence of any mold growth, as presented in Figure 6.

Careful observations were made on the 25th of July. It was noted that the growth of fungi exhibited an irregular distribution pattern when the relative humidity was 40% and the room temperature was 18.4 °C. Among the notable instances, one can point to the back side of the samples marked as (1A) and (4D), both of which were assigned a rating of 3 due to the significant extent of their fungal growth. Furthermore, it was observed that sample (2B) displayed limited growth, leading to its classification as a level-2 rating. Conversely, samples (3C), (5E), and (6F) had no fungal growth, resulting in their designation as a rating of 0, as demonstrated in Figures 7–9.

The microscopic examination of fungal growth of materials labeled (1A), (2B), and (4D) was carried out at the end of week 7 to gain detailed insights. It was noted that a substantial amount of fungal growth was observed on mixtures (1A) and (4D), compared to the mixture type (2B), which showed a minimal amount of such growth. This clear distinction was visually evident and was further substantiated by the data presented in Figure 10. These empirical findings corroborated the visual observations made on the same samples.

It is worth noting that the microscopic analysis did not reveal any evidence of fungal growth on the mixture type (3C). After the laboratory test, the optimized stabilized mixture type (3C), which includes 50% soil, 15% acrylic-based additive, 15% quicklime, and 20% Portland cement, was employed to construct an experimental rammed earth building within the designated study area. This mixture also yielded the highest compressive strength of 4.39 MPa according to laboratory structural examinations at an earlier phase of the current study [41]. The building consists of three rooms, each with a wall width measuring 40 cm and a total height measuring 4 m. The building was completed in August 2022.

Date of Observation	Mid of Week 2 (16th June)	End of Week 2 (20th June)	Mid of Week 3 (24th June)
Time	11:51 a.m.	10:56 a.m.	12:16 p.m.
Relative Humidity	50%	46%	45%
Room Temperature	18.7 °C	19 °C	18.3 °C

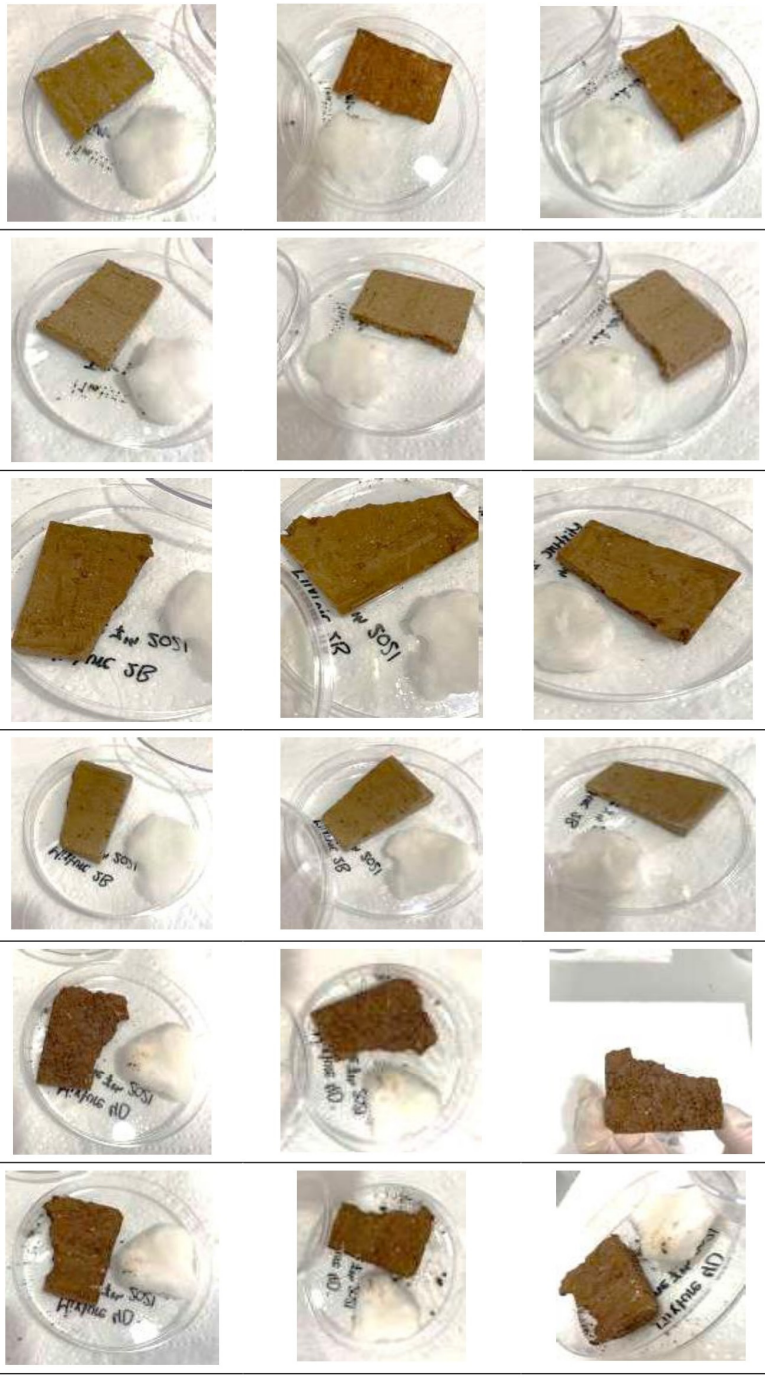


Figure 5. Macroscopic changes on the surface of the samples (1A), (2B), and (4D) during weeks 2 and 3.

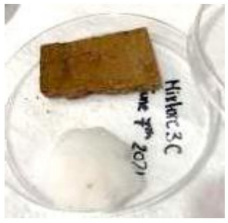
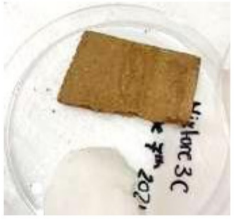
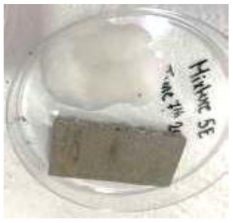
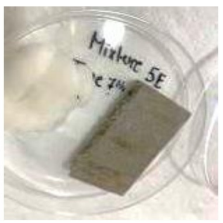
Date of Observation		Mid of Week 2 (16th June)	End of Week 2 (20th June)	Mid of Week 3 (24th June)
Time		11:51 a.m.	10:56 a.m.	12:16 p.m.
Relative Humidity		50%	46%	45%
Room Temperature		18.7 °C	19 °C	18.3 °C
Mixture (Type 3C)	Front			
	Back			
Mixture (Type 5E)	Front			
	Back			
Mixture (Type 6F)	Front			
	Back			

Figure 6. Morphological changes on the surface of the samples (3C), (5E), and (6F) during weeks 2 and 3.

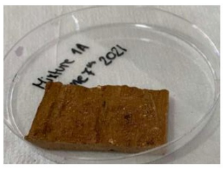
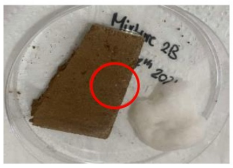
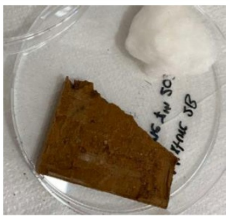
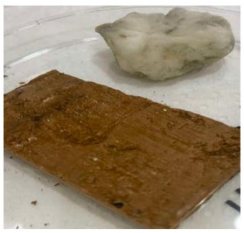
Date of Observation		Start of Week 1 (7th June)	End of Week 7 (25th July)
Time		12:30 p.m.	12:45 p.m.
Relative Humidity		45%	40%
Room Temperature		18 °C	18.4 °C
Mixture (Type 1A)	Front		
	Back		
Mixture (Type 2B)	Front		
	Back		
Mixture (Type 3C)	Front		
	Back		

Figure 7. Detailed fungal growth on materials (1A), (2B), and (3C) at the start and end dates of the laboratory test.

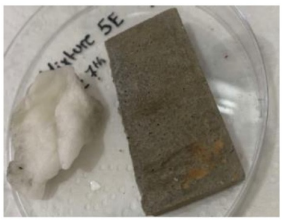
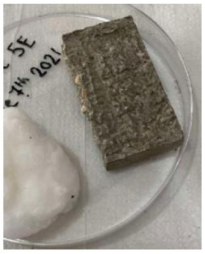
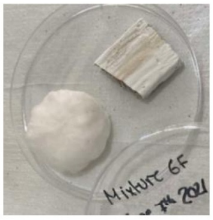
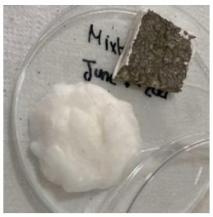
Date of Observation		Start of Week 1 (7th June)	End of Week 7 (25th July)
Time		12:30 p.m.	12:45 p.m.
Relative Humidity		45%	40%
Room Temperature		18 °C	18.4 °C
Mixture (Type 4D)	Front		
	Back		
Mixture (Type 5E)	Front		
	Back		
Mixture (Type 6F)	Front		
	Back		

Figure 8. Detailed fungal growth on materials (4D), (5E), and (6F) at the start and end dates of the laboratory test.

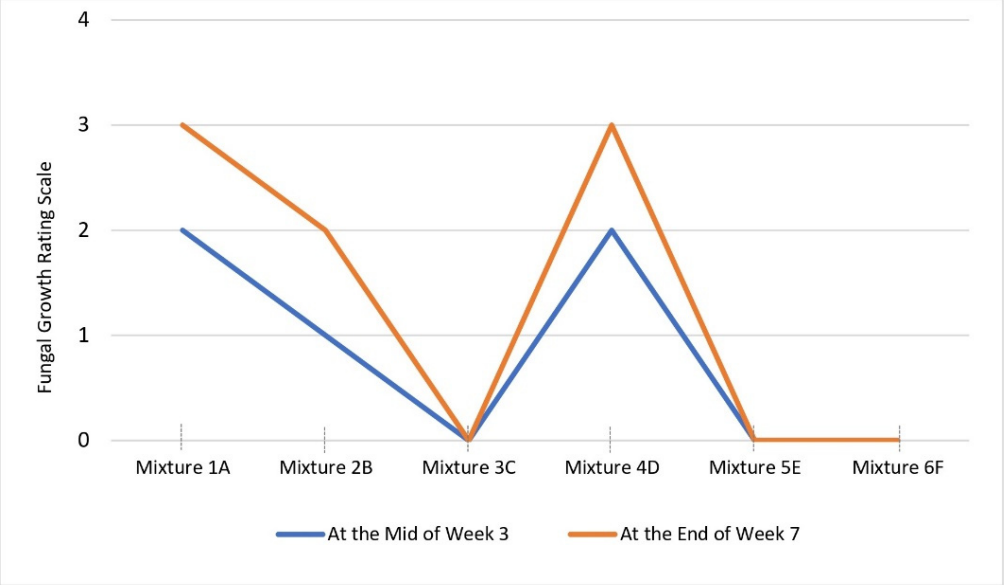


Figure 9. Rating scale of fungal growth on materials at the middle of week 3 and the end of week 7 of the laboratory test.

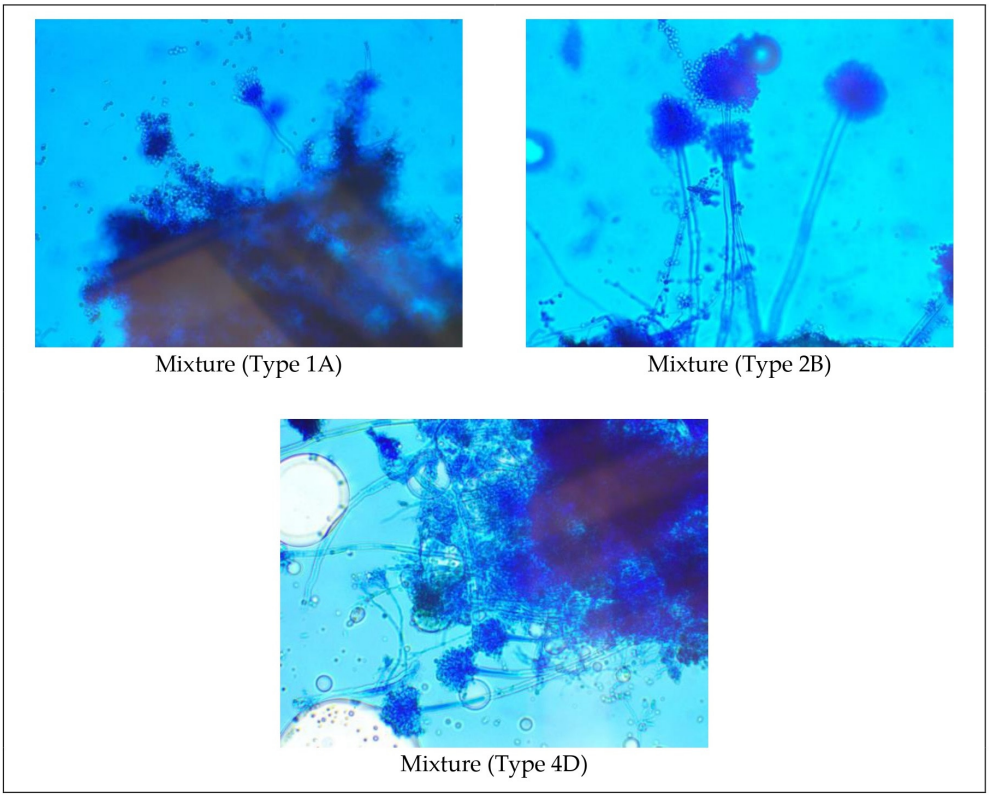


Figure 10. The microscopic observation of fungal growth in week 7.

During the winter season, between December 2022 and March 2023, an environmental post-occupancy study was conducted to assess fungal growth based on the achieved thermal conditions. Readings of the indoor air temperature and relative humidity levels inside the building are presented in Table 5.

Table 5. Air temperature and relative humidity conditions of the constructed building.

	December 2022		March 2023	
	Indoor Air Temperature	**Indoor Relative Humidity**	**Indoor Air Temperature**	**Indoor Relative Humidity**
Room 1	26.1 °C	38.4%	23.2 °C	38.7%
Room 2	26.7 °C	35.8%	23.5 °C	34.4%
Room 3	27.9 °C	34.7%	24.1 °C	35.2%

Based on the extensive examination conducted during that specific timeframe, findings indicated that the optimized combination employed did not exhibit any signs of fungal proliferation on the inner walls, as depicted in Figure 11.

Figure 11. External and internal views of the constructed rammed earth building.

4. Discussion

Although straws or plants were used historically in earth construction materials, this research did not utilize such additives, as they are significant causes of fungal growth due to the carbon and cracks which may increase moisture [35,39,40].

Laboratory tests were conducted on six mixtures under conditions found in inhabited buildings in hot–arid regions, where fungal growth is expected. The proposed methodology suggested an artificial incubation of fungi cultured from existing moldy walls. The procedure started with decontamination by exposing samples to high temperatures. Although previous studies suggested more effective methods to remove mold, such as gamma rays [36], the decontamination method was helpful in terms of time and cost.

After that, fungi were inoculated and added to the samples, then placed under a consistent relative humidity factor (45%) achieved using wet cotton wool. The incubation of samples lasted seven weeks by regulating the room temperature to fall between 18 °C and 19 °C and maintaining a controlled humidity level between 45% and 50%. These specific parameters were selected due to their suitability in fostering the growth of fungi within the study area and to ensure similar indoor environmental conditions in typical buildings in Jordan.

Although natural inoculation represents actual circumstances in buildings, artificial sourcing of fungal cultures from moldy walls is faster and is preferred by scholars [17,43].

This requires incubating cultured samples for a week at room temperature (24 °C) and a constant relative humidity of 45%. However, placing wet cotton wool near the samples and spraying it regularly can maintain the required humidity.

The incubation time of 7 weeks was enough to determine the fungal growth; although, several studies determined a range of 6 to 30 weeks to implement the test [17,36]. The results showed that fungi grew more efficiently on soil mixtures, including higher percentages of soil and lower percentages of additives. Mixture type (1A) consists of 80% soil, 5% acrylic-based additive, and 15% quicklime; mixture type (4D), was composed of 100% soil; and mixture type (2B), consists of 65% Soil, 15% acrylic-based additive, and 15% cement, and they all approved this claim. Mixtures (1A) and (4D) scored the highest rate of fungal growth; however, they had a lower intensity of fungal growth on the surface of mixture type (2B).

Mixtures that were stabilized with both quicklime and ordinary Portland cement revealed they were the best choice of soil mixtures. These additives could reduce the cracks and roughness of surfaces, so water accumulation would not be a source of mold growth [44]. These results agreed with previous studies that using Portland cement enhances materials' durability against water erosion and reduces surface degradation [26,28,45]. Furthermore, adding 15% of acrylic-based additives can protect the mixture against dampness [29].

Compared to the most used concrete mixtures in contemporary buildings, the study showed that the optimized mixture, type (3C), composed of 50% soil, 15% acrylic-based additive, 15% quicklime, and 20% Portland cement, can be used as a green, efficient replacement. No fungal growth was observed on the surface of the mixture. Moreover, the post-occupancy evaluation of the constructed building using mixture type (3C) proved that no fungal growth was spotted on the interior walls because of the environmental comfort conditions achieved by the utilized rammed earth materials. Although it is essential to perform the tests in high humidity conditions close to 90% [17], this setting is challenging to obtain and maintain in hot–arid regions. A future study in which proper funding is available would allow such an experiment. However, the humidity values studied cover the humidity range in residential buildings in the study area, based on the profile of environmental readings from previous studies.

5. Conclusions

This study investigated mold growth on the surface of earth constructions in the hot–arid region of Jordan. In addition, it explored the impact of adding cement, limestone, and a fast acrylic-based bond to the mixture on fungal growth resistance. Observations after seven weeks, under a regulated room temperature ranging between 18 °C and 19 °C, and a controlled humidity level between 45% and 50%, which covers the environmental conditions of residential buildings in the study area, showed that the growth of fungi was exhibited on mixtures that included higher percentages of soil and lower percentages of additives. Mixtures stabilized with quicklime, ordinary Portland cement, and acrylic-based additives were revealed as the best soil mixtures, which showed no fungal growth. The sample composed of 50% soil, 15% acrylic-based additive, 15% quicklime, and 20% Portland cement was put to the test in a rammed earth construction project and proved to be suitable for use as a green, sustainable, and efficient replacement after post-occupancy evaluation.

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