MORTAR DE TENCUIALĂ CU PROPRIETĂŢI ANTIBACTERIENE ŞI ANTIFUNGICE
PLASTERING MORTAR WITH ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES

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This study was focused on the development of plastering mortars with good antibacterial and antifungal properties and adequate physical and mechanical characteristics regarding the mechanical strengths (CS IV), good adhesion to the substrate and low water absorption by capillarity (W2). As testing microorganisms, four bacterial (Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa) and two fungal species (Aspergillus niger and Penicillium chrysogenum) as suspensions with density 10^6 cells/ml were used. In order to obtain antibacterial and antifungal effect on mortar surface, concentrations of more than 5% Ag/ZnO nanopowders (0.05 wt.%) are required. As a result, the obtained plastering mortars can be used for inhibiting the growth of pathogens of environmental and hygienic concern.

Acest studiu s-a axat pe realizarea de mortare de tencuială cu bune proprietăţi antibacteriene şi antifungice şi caracteristici fizice şi mecanice adecvate privind rezistenţele mecanice (CS IV), o bună aderenţă la substrat şi absorbţie redusă de apă prin capilaritate (W2). Au fost folosite suspensii (densitate 10^6 celule/ml) de patru specii de bacterii (Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa) şi două specii de fungi (Aspergillus niger and Penicillium chrysogenum). Pentru efect antibacterian şi antifungic la suprafaţa mortarelor sunt necesare concentraţii mai mari de 5% nanopulberi de Ag/ZnO (0.05 % gr.). Ca urmare, mortarele de tencuială obţinute pot fi folosite pentru inhibarea creşterii agenților patogeni de interes pentru mediu şi igienă.

Keywords: *mortar, zinc oxide, silver, antibacterial and antifungal properties*

1. Introduction

The quality of the indoor environment is a determining factor for health, given that people spend most of their lives inside buildings. Microbes and allergens, the absence of oxygen, inadequate temperature and humidity, mould, dust, improper lighting, ventilation and air currents, noise, the presence of building materials that contain noxious agents and generate toxic emissions, the functioning of equipment, and particularly pollution severely affect the health of people [1, 2].

Colonization of walls by different microorganisms (microalgae, fungi, lichens and mosses) can be observed even several months after a building is constructed. These microorganisms may spread to the surface of interior building materials under certain conditions of temperature, humidity (water flow and/or high relative humidity) and sunshine [2, 3]. Microorganism growth and spread is a major concern in various places, which require a high level of hygiene as medical products, packaging materials or filters used in air-conditioning systems [1]. Hospitals, pharmaceutical production units, nursing homes, food factories and shops require disinfection to destroy pathogenic microbes and other bacteria of public health [3-7].

In order to obtain antimicrobial concrete, mortar and coating systems, both organic and inorganic agents have been used: antimicrobial phenol derivatives for concrete-based floors and wall coverings [8, 9], polypropylene fibers treated with a combination of biocide and fungicide agents for concrete built agricultural premises for the inhibition of the growth of *Aspergillus niger*, *Staphylococcus aureus* and *Escherichia coli* [6], isothiazoline and carbamate for mortars [10]. A concentration of 1% zeolite with silver and copper ions relative to the cement weight is necessary for the inhibition of the growth of *Thiobacilli* and a variety of pathogens including *Escherichia coli*, *Staphylococcus aureus and Pseudomonas aeruginosa* [11], different concentrations of silver copper zeolites in mortars for inhibiting the growth of various pathogens of environmental concern

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(*Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica* or *Staphylococcus aureus*) [12].

Nanoparticles seem to be a suitable option for antimicrobial additives due to their small sizes and high surface to volume ratio, which allows them to interact closely with microbial membranes [13]. Up to now, researchers have focused their attention on the use of some metal oxides (TiO_2 [14], SiO_2 [14], MgO [15], CaO [16], CeO_2 [17]) and ZnO [14-18]) because these show bacteriostatic, antimicrobial or biocide action [19] and lower toxicity compared to other additives, as well as good heat resistance.

In addition to the above mentioned metal oxides, ZnO nanoparticles stand out due to their important characteristics: chemical and physical stability, high catalyzing capacity, antibacterial activity (against gram-negative bacteria such as *Bacillus subtillis*, *Esherichia coli* and *Pseudomonas fluorescens* and gram-positive bacteria such as *Staphylococcus aureus*) [20, 21], as well as intense ultraviolet and infrared absorption. ZnO has a broad range of applications, e.g., in catalytic, luminescent and electronic devices, pigments and components for the pharmaceutical and cosmetic industries [22-24].

ZnO powders doped with small silver quantity have good antimicrobial properties, so that these powders have been used as pigments with antimicrobial properties for indoor and outdoor painting [25]. Furthermore, chemical coprecipitation is an attractive method for preparing nanoparticles due to the good stoichiometric control, homogeneous mixing on the atomic scale and production of ultra-fine particles with narrow size distribution in relatively short processing time at low temperatures [26].

This paper presents the development of ecological plastering mortars with antibacterial and antifungal properties. The study is focused on the obtaining of plastering mortar with good adhesion to the substrate, low water absorption by capillarity, good mechanical, antibacterial and antifungal characteristics. The antibacterial and antifungal effects of those plastering mortars were investigated, using *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative) bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* (gram-positive) bacteria and two fungal species (*Aspergillus niger* and *Penicillium chrysogenum*).

2. Materials and methods

2.1. Plastering mortar specimens

The main materials used were white Portland cement 52.5, sand with particle sizes of 0-1 mm, water, and Ag-doped ZnO nanopowder (doping component in an amount of 0.05 wt.%) synthetized by coprecipitation method described in a previous publication [25]. The study was carried out on three plastering mortar compositions:

- Composition 1, M1 (standard) – unitary mortar based on white Portland cement 52.5 with 1:4 binder/sand ratio (particle size 0-1 mm) and water.
- Composition 2, M2 – the components of the standard composition were supplemented with a 5% Ag/ZnO nanopowder addition relative to the cement amount.
- Composition 3, M3 – the standard composition was supplemented with a 10% Ag/ZnO nanopowder addition relative to the cement amount.

The compositions of the mortars are shown in Table 1.

The mortars were prepared according to SR EN 1015-2:2001 [27]. The raw materials were weighed, homogenized in dry state, and subsequently mixed with water in the mixer.

Physical-mechanical determinations were performed after 28 days on prismatic specimens of 160mm x 40mm x 40mm, cast, compacted by vibration and stored during of this period according to standards SR EN 998-1:2011 [28], SR EN 1015-2:2001 [27] and SR EN 1015-11:2002 [29].

The consistency of standard mortar was determined with the flow table (Tecnotest, Italy), in conformity with SR EN 1015-3:2001 [30], meeting the requirement of SR EN 1015-2:2001 [27] according to which for fresh mortar with an apparent density higher than 1200 kg/m^3, the flow value is 175 ± 10 mm. To obtain the same consistency for the plastering mortars with Ag/ZnO nanopowder addition, it was necessary to increase the amount of water by 3.33% and 8.88%, respectively, parallel with the increase of the amount of nanopowder in composition with the consequence of a longer setting time.

The apparent density of hardened mortar was determined according to the SR EN 1015-10:2002 standard [31], the mortar prisms hardened for 28 days being were dried at constant mass and weighed.

Table 1

Studied mortars compositions / *Compoziţiile mortarelor studiate.*

Mortar indicative / *Indicativ mortar*	Components / *Componenți*				
	Cement / *Ciment*	Ag/ZnO nanopowder / *Nanopulbere Ag/ZnO*		Water / *Apă*	Sand 0-1 mm / *Nisip 0-1 mm*
	(g)	(%)	(g)	(ml)	(g)
M1	540	0	0	450	2160
M2	540	5	27	465	2160
M3	540	10	54	490	2160

The mechanical flexural and compressive strengths were determined according to the SR EN 1015-11:2002 standard [29]. The flexural strength was tested with the automatic flexural tensile tester (Controls, Italy) and the compressive strength with the 250 KN hydraulic press (Tecnotest, Italy).

The water absorption coefficient due to capillary action was determined according to SR EN 1015-18:2003 [32]. The prisms were broken in two pieces; they were dried at constant mass, sealed with paraffin along the long side, and immersed with the broken part in water for 5 mm up to 10 mm. After 10 min and 90 min, respectively, the prisms were taken out of the water, they were wiped and weighed, and the increase in the mortar mass was determined.

The adhesion of hardened mortar to the substrate was determined according to SR EN 1015-12:2001 [33], with the pull-off tester 58-C0215/T (Controls, Italy). Mortar was applied in a 10 mm layer to full bricks, which were previously humidified and were maintained in a vertical position during application. Adhesion to the substrate was also evaluated by the way of breaking: adhesive – breaking at the mortar-substrate interface or cohesive – breaking in mortar or in the substrate).

2.2. Antibacterial and antifungal efficiency

The 28-day-old-mortar specimens M1, M2 and M3 were cut into small prisms with dimensions of 40 mm x 40 mm x 1 mm. The mortar specimens were sterilized in a sterile laminar flow hood under ultra-violet illumination for 24 h at 22 ^{o}C. Mortar specimens were not exposed to autoclave conditions, in order to prevent significant alterations in the bulk mortar matrix and they were used immediately in the experiments.

The testing microorganisms used for this study were four bacterial species: *Escherichia coli* CCM 3954, gram-negative, *Staphylococcus aureus* CCM 4223, gram-positive, *Enterococcus faecalis* CCM 4224, gram-positive, Pseudomonas aeruginosa CCM 3955, gram-negative and two fungal species: *Aspergillus niger* CCM 8189 and *Penicillium chrysogenum* CCM 8034 - Czech Collection of Microorganism, Czech Republic.

Suspensions of cultures in 0.9% (w/v) NaCl solution were prepared (density 10^6 cells/ml, in fungi 10^6 spores/ml). The fungi were cultured on malt extract agar (AES Chemunex, France) and incubated for 2-5 days at 25^{o}C and bacteria - on nutrient agar no. 2 (Sigma-Aldrich, Germany) and incubated for 48 hours at 37^{o}C, respectively. The tested specimens were placed on Petri dishes with the appropriate cultivating medium and inoculated with 0.1 ml of the microbial suspensions using a sterile pipette. After incubation the plates were checked for zones of inhibition of bacterial and fungal growth. Each experiment was repeated at least three times with similar results.

2.3. Silver ions release

Mortar prisms having weight of approximatively 15 g were submerged in sterile centrifuge tubes containing 20 ml sterile TSB (Tryptic soy broth, Sigma-Aldrich, Germany), Dulbecco's modified eagle's medium (DMEM, Sigma-Aldrich, Germany) or ultrapure water for 1, 3, 6 and 24 h (three tubes for each sample). Subsequently, 10 ml of this solution was taken for the determination of the concentration of silver ions. The samples were digested with 2 ml HNO_3 and 1 ml H_2O_2 in a closed-vessel microwave system Berghof MWS-3+ with temperature control mode (Berghof, Germany). After cooling down to room temperature, the solutions were filtered and transferred into 25 ml volumetric flasks and diluted to the mark with deionized water. The silver content was determined by ICP-OES (OPTIMA 5300 DV, Perkin Elmer, USA). Each sample was measured for three times.

The calibration standards were prepared by appropriate dilution of the ICP multielement standard solution IV (Merck, Germany) 1000 mg/l. All reagents (HNO_3 65%, H_2O_2 30%) were of analytical grade and were purchased from Merck, Darmstadt, Germany. For all dilutions, ultrapure water (resistivity 18.2 MΩ/cm) obtained from a Millipore Direct-Q3 UV system (Millipore, France) was used. All PTFE and glass vessels were soaked in 10% HNO_3 for at least 24 h and rinsed extensively with Milli-Q water prior to use.

3. Results and discussions

3.1 Physical-mechanical characteristics

The following physical-mechanical characteristics were determined in the test specimens: the apparent density of hardened mortar, flexural and compressive strengths, water absorption by capillarity, and adhesion to the substrate. The results obtained by these determinations are synthesized in Table 2.

Table 2

Technical characteristics obtained / *Caracteristici tehnice obţinute.*

Mortar indicative / *Indicativ mortar*	*Apparent density* / Densitatea aparentă a mortarului întărit	Water absorption by capillarity / *Absorbţie de apă prin capilaritate*	Adhesion to the substrate / *Aderenţa*
	[kg/m^3]	[Kg/(m^2*min$^{0.5}$)]	[N/mm^2]
M1	1779	0.21	0.1
M2	1766	0.17	0.5
M3	1758	0.21	0.2

Additions of Ag/ZnO nanopowder will not significantly change the physical properties of the new mortars (M2 and M3) compared to the standard mortar (M1).

From Table 2, it can be observed that the apparent density of hardened mortars is little influenced by the Ag/ZnO nanopowder addition, although there is a slight decrease in apparent density with the increase in the amount of nanopowder. From the point of view of apparent density, all mortars join into the category of semi heavy mortars.

Regarding *water absorption by capillarity*, at a 5% Ag/ZnO nanopowder addition there is a decrease of water absorption by capillarity, while in the case of a 10% Ag/ZnO nanopowder addition, water absorption increases, reaching the value of standard mortar. The decrease in the water absorption capacity by capillarity is a positive factor, reducing the risk of dampness. The value obtained for mortar M2 shows a significant decrease in water absorption by capillarity (19%), compared to the standard mortar, which places mortar M2 in class W2. Standard mortar and mortar M3 takes part in class W1 in terms of water absorption by capillarity.

Mortar M2 is also the best regarding *adhesion to the substrate*. Its adhesion is 5 times higher than that of standard mortar (Table 2). Mortar M3 also has a 2 times higher adhesion to the substrate compared to the standard mortar.

As is shown in Figure 1, the mortar M2 shows a cohesive breaking - in mortar. M1 and M3 mortars show adhesive breaking, at the mortar-substrate interface.

Given that the studied plastering mortars contain less than 1% by mass or volume of homogeneously distributed organic materials, according to SR EN 998-1:2011 they are classified as fire reaction class A1, without needing to be tested.

Regarding mechanical strengths (flexural and compressive), an apparently strange behavior of mortars with Ag/ZnO nanopowder addition can be seen. At 2 days, these have a strength value tending to 0, while the flexural strength of standard mortar was 2.9 N/mm^2 and its compressive strength was 9 N/mm^2.

Only after 7 days, the mechanical strengths of mortars with nanopowder addition start to increase slowly. Thus, the flexural strength is 0.7 and 0.9 N/mm^2, respectively, which is much lower compared to that of standard mortar (3.4 N/mm^2), while compressive strength values are 0.5 and 1.5 N/mm^2, respectively, for standard mortar - 16.1 N/mm^2 (Figs. 2 and 3).

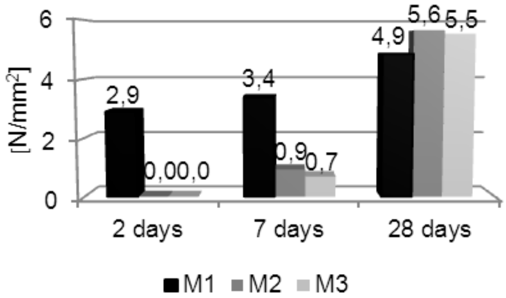


Fig. 2 - Flexural strength / *Rezistenţa la încovoiere.*

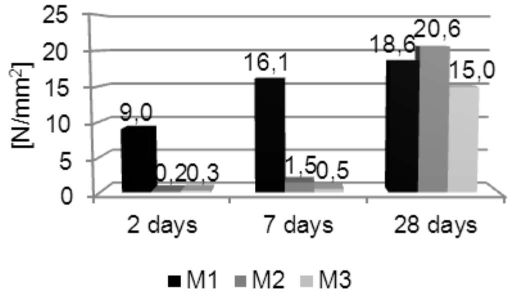
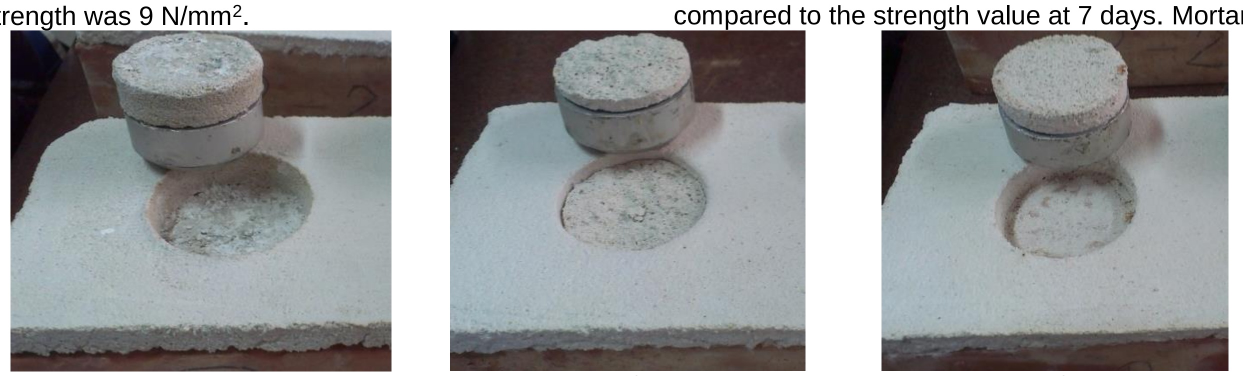


Fig. 3 - Compressive strength / *Rezistenţa la compresiune.*

At 28 days, both mortars with Ag/ZnO nanopowder addition have higher *flexural strength* values (5.5 and 5.6 N/mm^2), compared to standard mortar (4.9 N/mm^2).

Regarding the *compressive strength* of mortars with nanopowder additions, it can be seen that mortar with a 5% addition has a higher compressive strength compared to the standard mortar (20.6 N/mm^2 vs. 18.6 N/mm^2). Mortar with 10% Ag/ZnO addition has a lower compressive strength compared to the standard mortar (15.0 N/mm^2 vs. 18.6 N/mm^2), but this value is very high compared to the strength value at 7 days. Mortar



M1 M2 M3

Fig. 1 - Adhesion to the substrate / *Aderenţa mortarelor la stratul suport.*

Table 3

Amount of bacteria/fungi growth (%) on the mortar specimens containing Ag/ZnO nanopowders
Cantități de bacterii/fungi (%) dezvoltate pe specimene de mortar cu nanopulbere de Ag/ZnO.

Indicativ mortar/ *Mortar indicative*	***Escherichia coli (%)***	***Staphylococcus aureus (%)***	***Enterococcus faecalis (%)***	***Pseudomonas aeruginosa (%)***	***Aspergillus niger (%)***	***Penicillium chrysogenum (%)***
M1	100	100	100	100	100	100
M2	0	10	20	25	30	90
M3	0	0	0	0	15	75

M3, in terms of compressive strength takes part in class CSIV, like the other two types of mortar to which it was compared.

According to the obtained results, in terms of physical-mechanical characteristics, the optimal composition is that of M2 mortar containing 5% Ag/ZnO nanopowders; this has better mechanical characteristics than standard M1 mortar, as well as the best adhesion to the support layer and the lowest water absorption by capillarity. The increase in the added amount of Ag/ZnO nanopowders at 10% leads to a reduction of mechanical strengths. These results are similar with those reported by De Muynck et al.: a decrease in compressive strength of the mortars for higher concentrations of antimicrobial zeolites (4.65%) [12]. As a result, there is an upper limit for the amount of nanopowders that can be included in the composition of mortars without significantly affecting compressive strength.

3.2. Testing the antibacterial and antifungal efficiency

The antibacterial and antifungal efficiency of plastering mortars containing Ag/ZnO nanopowders and standard mortar without nanopowder is presented in Table 3. The antibacterial effect of M2 and M3 against bacteria *Escherichi*a *coli* and *Staphylococcus aureus* was excellent.

Plastering mortars have no inhibitory effect, and bacteria and fungi grew on them without restraint. The lowest concentration of Ag-doped/ZnO nanopowder in the plastering mortar was active against bacteria *Escherichia coli* and *Staphylococcus aureus*. Higher content of nano-Ag/ZnO completely inhibited all four bacteria growth and had an improved inhibition against *fungi Aspergillus niger and Penicillium chrysogenum,* due to the fact that nanoparticles may cause major damage to cell membranes and/or organelles to cause toxicity or cell death [26]. It is reported that, silver ions can cause bacterial penetration through the interaction with sulfur-containing proteins at the bacterial membrane, finally leading to the cell death [34]. Therefore, the combination of antibacterial and antifungal properties (of silver and ZnO nanoparticles) might be advantageous for the construction industry. The *Penicillium chrysogenum* fungus has proved to be very resistant, overgrowing almost the entire area of the coatings in all samples (level of the active substances in plastering mortar were insufficient for its inhibition). In order to obtain a whole antibacterial and antifungal effect on plastering mortar surfaces, concentration of Ag-doped ZnO nanopowders of more than 5% are required. Therefore, further experiments involving higher concentrations of Ag doped nanopowders in plastering mortars to improve the antifungal efficiency against *Aspergillus niger and Penicillium chrysogenum* should be tried.

The antibacterial and antifungal efficiency of Ag/ZnO demonstrated in our study is consistent with previous studies demonstrating antimicrobial effect of ZnO and silver ions [13, 23, 34-37], which make them promising for combating the growth of bacteria, fungi and algae on interior or exterior plastering mortar. The findings, however, have to be verified in the specific conditions (moisture, temperature, surface finish, etc.) to which these materials would be exposed in practical use (hospitals, institutional kitchens, nursing homes, athletic facilities, locker rooms, etc.).

3.3. Silver ions release

The study of silver ions release (different soaking times: 1, 3, 6 and 24 h) was performed in two culture media used in microbiological and cytotoxic assays, respectively TSB and DMEM. The critic soaking time was found to be 6 h: the amounts of silver ions released were about 1.0% in TSB and 1.2% in DMEM, respectively. As expected, for the specimen M1, no silver ions release was observed (<0.01 mg/l). The obtained results regarding the release of silver ion in TSB/DMEM compared to ultrapure water (Table 4) are in good agreement with those published in the literature: higher concentrations of silver ions in the mortar mixture resulted in a release of silver ions almost proportional to this increase. Kawahara et al. [37] reported that silver ions released from the silver zeolites require ionic exchange with other cations and it is dependent on the concentration of cations in the surrounding solution. Consequently, the silver ions are minimally released in deionized water or solutions with low ionic strength. Release of silver ions can be improved in the presence of sulfur-containing amino acids. More than 75% of silver ions were released from silver zeolites powders in brain heart infusion (BHI) broth after 30 min, with no additional release after 24 h incubation. On the contrary, after 24 h, no or low

Tabel 4

Release of silver ions (mg/l) from mortar specimens containing Ag/ ZnO nanopowders

Eliberarea ionilor de argint (mg/l) din probele de mortar cu nanopulberi de Ag/ZnO.

Mortar indicative *Indicativ mortar*	Leaching solution-Time *Timp de extracție*	**1 h**	**3 h**	**6h**	**24 h**
M2	UW	<0.010	<0.010	<0.010	<0.010
	TSB	0.101± 0.010	0.120 ± 0.012	0.181 ± 0.016	0.182 ± 0.014
	DMEM	0.123 ± 0.019	0.156 ± 0.011	0.225 ± 0.014	0.227 ± 0.014
M3	UW	<0.010	<0.010	<0.010	<0.010
	TSB	0.200 ± 0.011	0.243 ± 0.014	0.375 ± 0.016	0.372 ± 0.012
	DMEM	0.289 ± 0.012	0.310 ± 0.013	0.450 ± 0.011	0.449 ± 0.014

detectable amounts of silver ions were observed in deionized water and phosphate-buffered saline (PBS), respectively although the bactericidal activity was high. These results suggest that the bacterial cells in contact with silver zeolite absorb silver ions, which inhibit several functions in the cell and damage the cells [26].

The release of silver ions from the plastering mortar specimens has important relevance in their biocidal activity in long term. Successive releasing of silver ions results in the loss of the biocidal activity. Therefore, the antibacterial and antifungal activity is clearly dependent on environmental parameters. It is expected that antibacterial and antifungal mortar surfaces to have a longer activity in areas such as hospitals, institutional kitchens, nursing homes, athletic facilities, locker rooms, in comparison with wet areas such as shower, bathrooms and laundries.

The study demonstrates the possibility of obtaining a viable plastering mortar, with good antibacterial and antifungal properties. The technology for the development of the proposed material raises no special problems, given that the classical manufacture technology for plaster mortar is used, which requires no additional costs. The cost of the material is only affected by the cost of the Ag/ZnO nanopowder addition. Considering that the obtaining of these plastering mortars involves the use of small amounts of this nanopowder, the manufacturing costs are not significantly increased. As a result, the market price of this product will increase by approximately 3-4 %. Its properties and accessible price recommend it not only for the areas with particular hygiene requirements but also for other healthy indoor environments. Thus, these plastering mortars have good perspectives to enter on the building materials market.

4. Conclusions

In this study, a new antibacterial and antifungal plastering mortar containing small amount of Ag/ZnO nanopowders was obtained. From the physical-mechanical characteristics point of view, the optimal composition is that of mortar with 5% Ag/ZnO nanopowder addition. Through its characteristics, according to standards, this fits in class CSIV in terms of compressive strength at 28 days, and in class W2 regarding water absorption by capillarity. The flexural strength and adhesion to the substrate are also higher than the values obtained for the standard mortar.

Plastering mortars containing Ag-doped ZnO nanopowders exhibited excellent inhibitory effect against the growth of four tested bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*) and one fungi (*Aspergillus niger)*. However, even the highest amount of Ag-doped ZnO was not sufficient to inhibit fungi *Penicillium chrysogenum*. Such plastering mortars should be employed for the improvement of the hygienic conditions in a variety of environments such as hospitals, institutional kitchens, nursing homes, athletic facilities, locker rooms where antibacterial and antifungal properties are required.

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