ANTIMICROBIAL ACTIVITY OF GEOPOLYMERS WITH METAL MICROPARTICLE ADDITIVE

[1]Vojtěch RŮŽEK, [1]Lucie SVOBODOVÁ, [1]Totka BAKALOVA, [1]Martina RYVOLOVÁ

[1]*Department of Materials, Technical University of Liberec, Liberec, Czech Republic, EU,*
vojtech.ruzek@tul.cz

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Abstract

This paper investigates the geopolymers with metal microparticles (metal powder) as additives and assesses the antimicrobial effect on these composites. Samples of geopolymers were prepared with 4 % silver, copper, or nickel microparticle additive. The antimicrobial properties were based on a leaching test from composites, and the inhibition zone around *Escherichia coli* and *Micrococcus luteus* bacteria cells was investigated. Microparticles, especially silver and copper, were confirmed effective as antimicrobial reagents in geopolymer composites, with copper microparticles also being favourable over silver due to its significantly lower price.

Keywords: Geopolymer, antimicrobial property, bacteria cell, microparticle, metal

1. INTRODUCTION

Geopolymers are materials formed by the polycondensation of various precursors (such as fly ash [1] or metakaolin [2]) in a strongly alkaline environment [3]. They are a potential alternative to materials based on ordinary portland cement (OPC), which is the most widely used construction material and a base for concrete [4]. When compared to OPC based materials, geopolymers have higher compressive strength [5], resistance against high temperatures [6], lower thermal conductivity [7] etc. However, like OPC based materials [8], geopolymers are also susceptible to microbially induced degradation (MIB) [9], especially in wet or humid environment. Geopolymers exhibit significant antimicrobial properties in dry state due to their high alkalinity. In humid environment, the geopolymer or concrete surfaces may be colonized by alkali-resistant bacteria species (usually sulfur-oxidizing bacteria [9]). These bacteria produce acidic compounds (like hydrogen sulfide or sulphuric acid), causing degradation of the material and allowing colonization by other microorganisms (including other bacteria, fungi, lichen [10], and algae [11]).

This study used silver, copper, or nickel microparticles (in the form of a powder) instead of metal nanoparticles. Their antimicrobial effect in geopolymer was investigated as a potential alternative to nanoparticles and other antimicrobial agents (while being effective, nanoparticles may pose a serious risk to the environment and are more expensive). The antimicrobial effect was tested on both gramnegative (*Escherichia coli*) and grampositive (*Micrococcus luteus*) bacteria.

2. MATERIALS AND METHODS

2.1. Geopolymer Samples Preparation

The geopolymer samples were prepared using locally sourced Baucis L_K metakaolin-base and potassium-based activator, both manufactured by ČLUZ, a.s Czech company [12]. In addition, three types of microparticles were used to prepare geopolymer samples, namely silver, copper and nickel. The microparticle size was: silver (made by PkChemie) and copper (made by Fischema) were both sized below 45 μm in diameter, and nickel microparticles (made by Selkat ireneusz Katarzynski) in the range of 3-7 μm. Each set of samples contains one type of microparticle, except for the control sample. The composition of geopolymer samples is shown in **Table 1**.

Table 1 Geopolymer samples composition (materials are listed in weight proportion to the geopolymer base).

Material	Weight proportion
Geopolymer base	100
Activator	90
Microparticles	4

After hardening, the samples were cut to roughly 3x3x1 cm samples and analyzed on confocal microscope S neox, manufactured by Sensofar, with 20x magnification and image mode (without 3D surface mapping). It was confirmed that microparticles are dispersed in geopolymer matrix, as they were visible on the surface. The confocal images are shown in **Figure 1**.

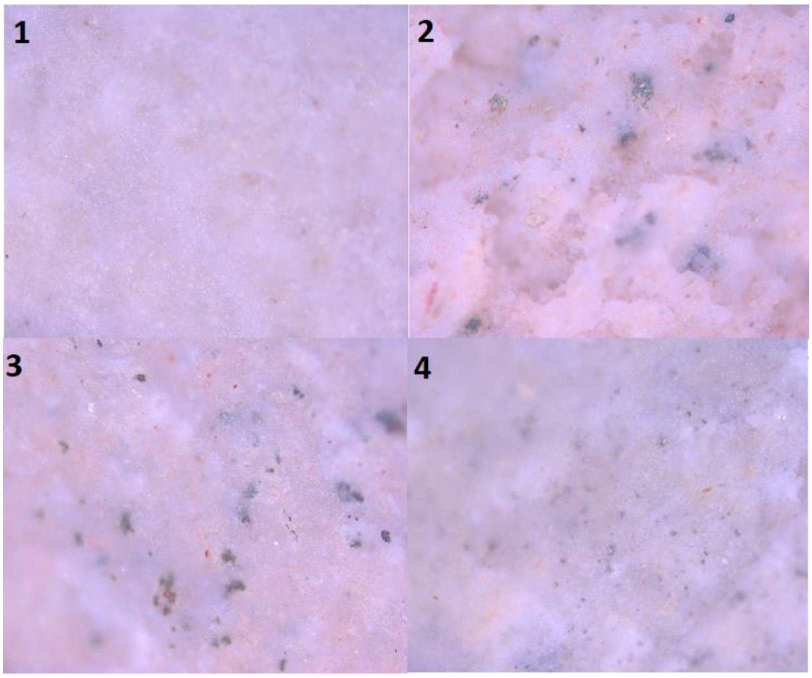

Figure 1 Images of geopolymer sample surface with 20x magnification, 1 = Pure geopolymer, 2 = Geopolymer with silver microparticles, 3 = geopolymer with copper microparticles, 4 = geopolymer with nickel microparticles

2.2. Antimicrobial Activity Assessment Tests

Gram-negative *Escherichia coli* and gram-positive *Micrococcus luteus* bacteria cells were bought from the Czech microorganism's collection [14] and were incubated for 24 hours at 37 °C before assays. Bacterial inoculum was prepared with a concentration of 10^5-10^6 CFU/mL (Colony forming units per milliliter), applied on an agar (Mueller-Hinton) surface in the Petri dish, and spread using a glass spreader. The bacteria cells were incubated in an agar medium for 15 minutes at 25 °C. The antimicrobial activity was investigated using a quantitative disk diffusion test in an agar medium (modified Kirby-Bauer test). The tested samples were applied in the middle of the Petri dishes and incubated for 24-48 hours (by microorganism type); each measurement was repeated four times.

Two types of antibiotics (Cefazolin 30 µg and Gentamicin 10 µg) were used as a positive control (with expected inhibition) in the form of antibiotic-infused paper discs; pure bacteria cells in saline solution were used as a negative control sample (with no inhibition). The antimicrobial activity of geopolymers with added microparticles was tested in the form of leach in saline solution (0,9 % NaCl). The leachate was prepared five days in advance. The leachate properties are specified in **Table 2**.

Table 2 Geopolymer leach (a geopolymer with no microparticles was used as a control sample).

Sample	Geopolymer weight (g)	Saline solution volume (ml)	Weight/volume ratio (%)	pH (t=0)	pH (t=5 min)
Control (1)	17.90	179	10	6.99	9.93
Ag (2)	15.02	150	10	6.99	9.65
Cu (3)	20.88	208	10	6.99	9.23
Ni (4)	22.65	227	10	6.99	9.09

The microparticle leachates were tested in a saline solution prepared five days before testing. In this medium, silver microparticles quickly sedimented (under 5 minutes) and significantly agglomerated even after thorough stirring. The agglomeration and sedimentation rate of copper microparticles was lower than silver microparticles. Nickel microparticles did not agglomerate and sedimented slowly (presumably due to their smaller size). The prepared mediums are specified in **Table 3**.

Table 3 Microparticle medium.

Microparticle	Weight (g)	Saline solution volume (ml)	Weight/volume ratio (%)	pH (t=0)
Ag (a)	0.317	13.17	2.41	6.99
Cu (b)	0.305	13.05	2.34	6.99
Ni (c)	0.620	16.20	3.83	6.99

The antimicrobial activity is proved when a visible inhibition zone around the tested area exists; the area without cells (colony forming units) may be easily measured. Image analysis software ImageJ (the National Institute of Health) was used to measure the diameters and areas of the inhibition zones. In each image, the background and agar color was changed to black, while bacterial colonies were colored to the scale of brown (to allow easy comparison and measurement of inhibition zones). Due to inhibition zones not always being perfectly circular, the area was measured instead of a diameter, and the average diameter was calculated from the measured area (according to the equation for the circle's volume). Basic statistical analysis (average values, standard deviation, exclusion of outliers) was calculated. Example images of inhibition zones are shown in **Figure 2**.

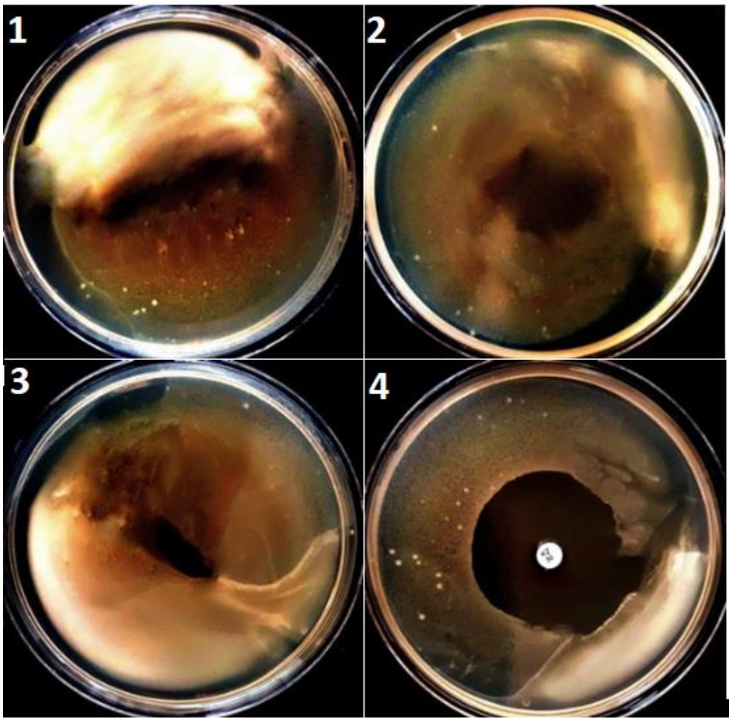

Figure 2 Inhibition zones for *Escherichia coli*; 1 = Silver microparticles geopolymer leach, 2 = Copper microparticles geopolymer leach, 3 = Nickel microparticles geopolymer leach, 4 = Cefazolin 30 μg

3. RESULTS AND DISCUSSION

Figure 3 shows the diameters of inhibition zones for each sample, while **Figure 4** shows antimicrobial activity of samples as a percentage value relative to the activity of the antibiotic (Cefazolin 30 µg). The positive control samples (Cefazolin or Gentamicin) proved the highest average antimicrobial activity (diameter of inhibition zones). In contrast, the negative control samples (pure bacteria cells in saline solution) showed no effect, per expectations.

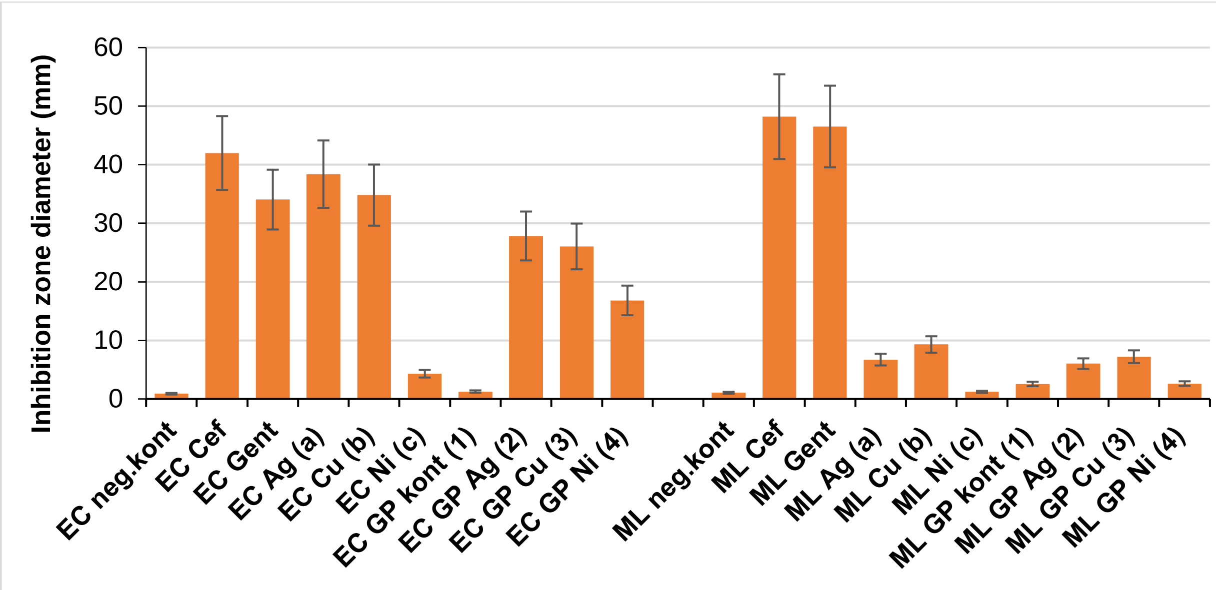

Figure 3 Inhibition zone diameters. EC = *Escherichia coli*, ML = *Micrococcus luteus*; neg.cont = Negative control sample; Cef = positive control sample Cefazolin 30 µg; Gent = Positive control sample Gentamicin 10 µg. Marked identically to Tables 2 and 3.

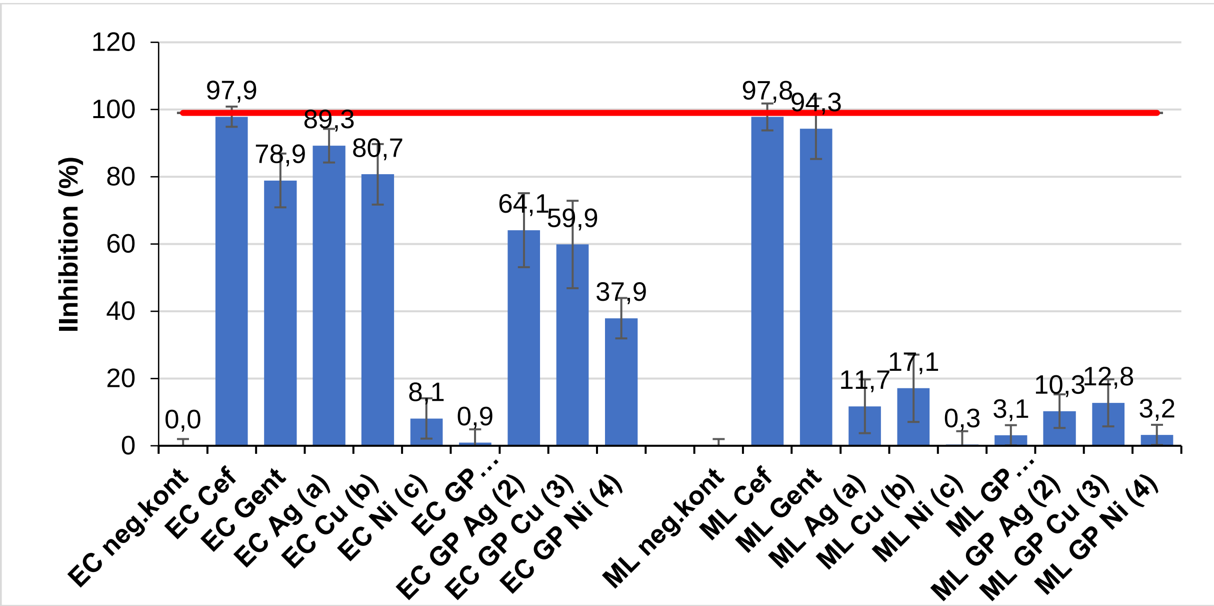

Figure 4 Graph of inhibition effect of antimicrobials compared to antibiotics; EC = *Escherichia coli*, ML = *Micrococcus luteus*; neg.cont = Negative control sample; Cef = positive control sample Cefazolin 30 µg; Gent = Positive control sample Gentamicin 10 µg. The red line in the graph shows 100% antimicrobial activity. Marked identically to Tables 2 and 3.

For *Escherichia Coli* (gram-negative bacteria cells), silver and copper microparticles in the geopolymer matrix (in **Figure 1** marked as EC GP Ag (2) or EC GP Cu (3)) have shown a high antimicrobial activity of up to 64 % and 60 %. Pure silver microparticles in saline solution (in **Figure 1** marked as EC Ag (a)) had a slightly higher antimicrobial activity than copper microparticles (EC Cu (b)) by about 9 %, although within a margin of error. The antimicrobial activity of pure nickel microparticles (EC Ni (c), despite their smaller size) is much lower, reaching only 8 %. In the geopolymer matrix (EC GP Ni (4)), these particles' effect is almost 38 % (it may be a synergistic effect of particles and high pH). Despite its high pH, pure geopolymer leach (EC GP kont (1)) does not affect *Escherichia Coli* growth.

For *Micrococcus Luteus* (gram-positive bacteria cells), the antimicrobial activity of microparticles was much weaker compared to *Escherichia Coli,* both in pure form and as incorporated in the geopolymer matrix (in **Figure 1** marked as ML). Copper microparticles have achieved the highest antimicrobial activity, about 17 % in saline solution (ML Cu (b)) and almost 13 % in geopolymer matrix ((ML GP Cu (3), relative to the Cefazolin). Nickel microparticles (pure ML Ni (c) or in geopolymer matrix ML GP Ni (4)) and pure geopolymer leach (ML GP kont (1)) did not affect *Micrococcus Luteus* growth (values are lower than 5 %).

Results indicate that microparticles, especially silver and copper, may be an appropriate alternative to nanoparticles to reach a high antibacterial activity (even as additives in geopolymer). While the inhibition effect on *Micrococcus luteus* was much lower than the effect on *Escherichia coli*, microparticles inhibited bacterial cell growth all the time. Microparticles may prevent the colonization of the geopolymer matrix surface even against gram-positive bacteria, which is a key factor in preventing microbially-induced degradation. The disadvantage at present is that the microparticles may leach out of the geopolymer matrix.

The nickel microparticles reached higher antimicrobial activity as part of geopolymer composite than by themselves. It is possible that, while weak on their own, nickel microparticles and alkaline geopolymer environment have a synergistic effect, giving nickel microparticle geopolymer composite higher antimicrobial activity than its components. However, it may also indicate higher rate of leaching out of microparticles into the environment. As such, leaching rate of microparticles should be tested in further studies. However, despite the improved antimicrobial effect of nickel microparticle geopolymer composite, the antimicrobial activity of silver and copper microparticles geopolymer composites was still significantly higher when compared to nickel microparticle geopolymer composite.

Despite their antimicrobial effectiveness, silver microparticles are significantly more expensive than other metal microparticles, including similarly effective copper. Silver microparticles were bought for around 1850 EUR/kg, while copper microparticles were bought for around 75 EUR/kg. With current data, the significant difference in price makes copper microparticles the best choice from the three tested types, due to their high antimicrobial activity and relatively low price. However, it is currently unknown whether copper or silver microparticles antimicrobial activity would persist with lower microparticle content in geopolymer matrix, as high antimicrobial activity of low silver microparticle content may justify their use as antimicrobial agent in geopolymers, despite higher price.

4. CONCLUSION

This paper investigated silver, copper and nickel microparticles as a potential antimicrobial additive to geopolymers. The high antimicrobial activity of silver and copper microparticles was confirmed by testing (a) pure microparticles in saline solution and (b) leaching test of geopolymer with microparticles as additives. Silver microparticles are, however, very expensive, making copper microparticles more suitable as antimicrobial geopolymer additive. Further studies will be focused on finding assessment options for the antibacterial effectiveness of these highly alkaline materials (testing the geopolymer sample surface itself instead of leach). The effect of microparticle content on mechanical properties and their release rate from the geopolymer matrix for environmental acceptability assessment will be investigated, as well as antimicrobial activity of geopolymers with lower microparticle content.

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