Preparation of Bactericidal Fillers from Georgian Heulandite-Clinoptilolite and Their Application for Paper Production. I. Bactericidal Fillers

Abstract. The purpose of present work was to obtain bactericidal materials using heulandite-clinoptilolite from the Rkoni plot of the Tedzami deposit, Eastern Georgia, to study their properties and use them as a filler for the production of bactericidal paper. Silver-, copper-, and zinc-containing microporous materials have been prepared using ion-exchange reactions between preliminary acid-treated zeolite microcrystals and a salt of a corresponding bioactive metal in the solid phase followed by washing with distilled water. Synthesized in such way adsorbent-ion-exchangers are characterized...
by X-ray energy dispersion spectra, powder X-ray diffraction patterns, and Fourier transform infrared spectra. Obtained materials remain the zeolite crystal structure and contain over 130 mg/g of silver, 70 mg/g of copper, and 55 mg/g of zinc. Prepared materials show bacteriostatic activity towards gram-negative bacterium Escherichia coli, gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, fungal pathogenic yeast Candida albicans, and a fungus Aspergillus niger. It was found that the mixtures of various forms exhibit a synergistic effect, and the silver form with additives of copper and zinc forms is most active against staphylococcus, and against other microorganisms, mixtures of copper and zinc forms are most effective.

**Keywords:** heulandite-clinoptilolite, bacteriostatic activity, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus niger.

According to reports of the World Health Organization, millions of deaths occurring worldwide are because of infectious diseases caused by bacteria, viruses, fungi and parasites, so interest in disinfectants is constantly increasing, especially in the face of the coronavirus pandemic. Research on the creation of new antibacterial and antiviral substances is carried out in many countries, along with liquid disinfectants, solid ones are being developed. In particular, germicidal powder materials can be used in the production of polymeric materials and paper as fillers to protect the surface of products from microbial contamination.

Among advanced materials, zeolites \( M_x[Al_{x-y}Si_yO_{2(x+y)}]mH_2O \), in which ions of alkali or alkaline-earth metals \( M \) are partially replaced by ions of a bioactive metal (\( Ag^+ \), \( Cu^{2+} \), \( Zn^{2+} \), etc.) are recognized as promising [1-3]. Studies that started at the beginning of the 21st century, continuing to this day and reflected in numerous publications, have shown that synthetic [4-22] and natural [14, 22-34] zeolites enriched with bioactive metals exhibit antimicrobial activity against a wide range of microorganisms. Most of the research concerned the study of silver-containing zeolites [4-6, 9, 11, 13, 15, 23, 25, 28], and as a result of studies of silver-, copper- and zinc-containing zeolites [7, 8, 10, 14, 21, 24, 26, 27, 29], it was found that the silver-containing zeolites are the most active. For example, silver-containing natural clinoptilolite and synthetic zeolite A are characterized by highest antibacterial activity against different isolates of gram-negative bacteria Escherichia coli, while copper-containing and, in particular, zinc-containing forms are inferior to them in
activity [14]. Zeolite-rich tuff from the State of Chihuahua, Mexico (ZCh) modified with Ag⁺ or Cu²⁺ ions showed microbicidal effect against *E. coli* and a yeast *Candida albicans*, but Cu-ZCh acts 20-30 times weaker than Ag-ZCh [29].

However, the disadvantages of the use of silver ions have been noted – silver is an expensive metal, and Ag⁺ is not stable in aqueous solutions, tends to be reduced to Ag⁰ and reacts with sulfate and other anions forming insoluble salts [32], so in some cases the benefits of silver ions are not so obvious. Thus, Zn²⁺ and Cu²⁺ loaded samples of synthetic zeolite X showed excellent antimicrobial activities against three bacteria – gram-negative *E. coli* and *Pseudomonas aeruginosa*, gram-positive *S. aureus*, a yeast *C. albicans* and a fungus *Aspergillus niger* [12]; according to the recent results [19], Cu-X is more active against *S. aureus*, Zn-X against *E. coli*, although the zinc form of synthetic zeolite A is inactive [14].

In most studies applying natural zeolites for preparation of bactericidal materials, heulandite-clinoptilolite of various origins was used [23-30], and results for bioactive metal-enriched clinoptilolites are also inconsistent. On the one hand, it was found that the diameters of the zones of inhibition of the growth of *E. coli* by the Ag-, Cu-, and Zn-forms of clinoptilolite from Görde, Turkey, at a exchange level of ~0.2 are approximately the same and amount to 12 mm, the maximum zone of inhibition by Ag⁺ ions is 14 mm, and by Cu²⁺ ions – 13 mm [24]. On the other hand, natural clinoptilolite from Mare Baia, Romania (CLI, [34]) was “activated” by Cu²⁺ and Zn²⁺ ions and tested on antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans*, but only Cu-CLI and only against *E. coli* appeared to be active [30].

The purpose of this work was to obtain bactericidal materials using natural heulandite-clinoptilolite from the Rkoni plot of the Tedzami deposit, Eastern Georgia, to study their properties and use them as a filler for the production of bactericidal paper.

**Characterization of heulandite-clinoptilolite**

Heulandite-clinoptilolite is one of the most widespread in Nature, well studied and widely used zeolites [35], its crystals are distinguished by their characteristic monoclinic appearance; idealized cell data: space group *C2/m*, \(a = 17.5\,\text{Å}, \, b = 17.6\,\text{Å}, \, c = 7.4\,\text{Å}, \, \beta = 116.1^\circ\) [36]. The framework (Fig. 1, left) is characterized by two-
dimensional system of open channels formed by 10- and 8-membered rings; channels parallel to [001] with elliptical 10- and 8-ring (Fig. 1, middle) apertures are inter-connected at right angles by one-dimensional system of smaller 8-ring channels (Fig. 1, right) parallel to [100].

Fig. 1. Heulandite-clinoptilolite framework, 10- and 8-membered rings viewed along [001], and smaller 8-rings viewed along [100]

Heulandite-clinoptilolite belongs to the HEU group with ideal chemical formula of the unit cell $\text{Ca}_4(\text{H}_2\text{O})_{24}[\text{Al}_8\text{Si}_{28}\text{O}_{72}]$, but the chemical composition of its varieties is characterized by remarkable changes in the Si/Al ratio (from 2.7 to 5.5) as well as in the content of exchangeable cations [37].

Heulandite-clinoptilolite-bearing rock from the Rkoni plot of the Tedzami deposit has zeolite phase content of 90% and chemical composition described by averaged empirical formula of dehydrated zeolite ($\text{Na}_{0.25}\text{K}_{0.06}\text{Ca}_{0.19}\text{Mg}_{0.15}$) $[\text{AlSi}_{3.6}\text{O}_{9.2}]$ calculated from the X-ray energy dispersive (XRED) spectroscopy data (Fig. 2) obtained using an Oxford Instruments X-Max 20 analyzer.

Fig. 2. SEM image and typical XRED spectrum of raw heulandite-clinoptilolite
In addition, XRED spectra show the presence of titanium atoms and impurities containing calcium and iron. The titanium content is one atom per 240±20 aluminum and silicon atoms, and since this value remains unchanged for ion-exchange samples, there is reason to believe that titanium atoms are part of the zeolite crystal lattice. On the contrary, atoms of calcium and iron compose amorphous or crystalline impurity inclusions visible in the layered XRED images (Fig. 3).

![Layered XRED images of calcium (left) and iron (right) distribution](image)

The crystal structure and properties of heulandite-clinoptilolite from the Rkoni plot are described in detail in our recent work [38]; the powder X-ray diffraction (XRD) pattern (modernized Dron-4 X-ray diffractometer employing the Cu-Kα line $\lambda = 0.154056$ nm, 2$\Theta$ range 5-70° at 1°/min) with assignment of some peaks is shown in Fig. 4 in comparison with simulated pattern from the collection [39].

The adsorption-desorption isotherms of nitrogen (measured at 77 K using ASAP 2020 Plus analyzer from Micromeritics, Norcross, GA, USA) on natural zeolite samples correspond to the filling of micropores (Langmuir plot) at low relative pressures ($p/p_0<0.3$) and demonstrate a hysteresis loop with a jump at $p/p_0=0.4-0.5$ indicating the presence of mesopores. The specific surface area calculated by the Brunauer-Emmett-Teller method ($S_{BET}$) ranges from 8.0 to 12.6 m$^2$/g, and the total pore volume from 0.71 to 0.85 cm$^3$/g, respectively; for samples with high $S_{BET}$, average diameter of mesopores, calculated by the Barrett-Joyner-Halenda method using adsorption and desorption isotherms, is 24.6 and 15.7 nm, respectively.
Fig. 4. Powder X-ray diffraction pattern of the Rkoni zeolite (a, numbers in parentheses are Miller indices) compared with simulated [39] pattern (b)

Acid treatment (0.01 – 1N) leads to a significant increase in the specific surface area (up to 190 m²/g) with a slight increase in the total pore volume (up to 0.138 cm³/g), while a significant decrease in the diameter of mesopores is observed (up to 8.9 and 10.6 nm using adsorption and desorption isotherms, respectively). Treatment of zeolite with dilute solutions of hydrochloric acid also leads to an increase in ion-exchange capacity up to its scientific weight value $C_W$ expressed in milliequivalents per gram of the heulandite-clinoptilolite completely converted to the hydrogen form $(H^+(H_2O)_3[AlSi_{3.6}O_{9.2}])$ [40].

For all further experiments, the rock was crushed in the planetary micro mill Pulverisette 7 (Fritsch Laboratory Instruments, Idar-Oberstein, Germany) to a size less than 0.044 mm (325 US mesh), crushed and sieved rock was washed by diluted HCl solution (0.025 N) to remove clay impurities and improve ion-exchange properties, and named as HCR (heulandite-clinoptilolite from Rkoni).

According to the value of the silicate modulus (Si/Al = 3.6), HCR should be attributed to the group of high-silica heulandites, but heulandites usually have a
cational composition with a predominance of calcium (Ca>Na>K), while sodium predominates in HCR like in low-silica clinoptilolites [37]. Moreover, the thermal properties of zeolite rather indicate its belonging to the clinoptilolite variety – dehydration occurs in the temperature range of 40-330 °C, endothermic peaks at 200 and 340 °C (formation of metaheulandite B) are not observed, but an endo-peak is recorded at 160 °C, and the sample retains its crystal structure at least up to 350 °C.

However, in the infrared spectrum collected by a 10.4.2 FTIR spectrometer (Perkin-Elmer, UK), the band of external asymmetric stretching vibrations \( v_{\text{asym}} \) has two maxima – one at 1065 cm\(^{-1}\), which is characteristic of clinoptilolites, and another at 1056 cm\(^{-1}\), which is close to the characteristic value for heulandites (1050 cm\(^{-1}\)). Absorption band of internal asymmetric stretching vibrations at 1200 cm\(^{-1}\) was recorded as a shoulder at broad band \( v_{\text{asym}} \), the rest of the bands have values typical of the HEU zeolite framework: internal and external symmetric stretching vibrations at 724 and 778 cm\(^{-1}\), respectively, as well as external tetrahedral deformational double ring vibrations at 520 and 600 cm\(^{-1}\), and internal tetrahedral bending vibrations at 450, 463, and 470 cm\(^{-1}\).

### Preparation of Ag-, Cu-, and Zn-forms of HCR

The preparation of metal-containing zeolites is usually carried out by treating the zeolite powder in an aqueous solution of the corresponding salt, recently it has been shown that the use of ultrasound ensures desorption of air from clinoptilolite particles and accelerates the diffusion of ions and subsequent ion exchange [41].

We recently obtained phillipsites, analcimes and synthetic zeolites A [22] containing silver, copper and zinc, and possessing a bacteriostatic effect against *E. coli*. These materials were obtained by the method of “solid” ion exchange, which was also used for preparation of bactericidal heulandite-clinoptilolites.

Ion exchange was carried out as follows: powder of HCR and the corresponding salt (analytical grade silver (I) nitrate AgNO\(_3\), copper (II) chloride dihydrate CuCl\(_2\)2H\(_2\)O, and zinc (II) chloride ZnCl\(_2\) purchased from Merck KGaA, Darmstadt, Germany, and used without any further purification) were mixed (molar and weight ratios are given in Table 1) and thoroughly grinded in an agate mortar for 10 minutes for AgNO\(_3\)-containing mixture, and for 15 minutes for CuCl\(_2\) or
ZnCl$_2$-containing mixtures. The solid mixture was then transferred to a filter and washed with distilled water until the complete disappearance of nitrate or chlorine anions, after which the modified samples were first dried in air and then at 100-105°C in a thermostat; samples are labeled as AgHCR, CuHCR, and ZnHCR (silver-, copper-, and zinc-containing heulandite-clinoptilolite, respectively).

Compared to the maximum possible biocide metal content, which follows from the previously published [24] data on ion exchange isotherms on clinoptilolite from Gördez, Turkey (1.8, 1.2, and 1.0 mmol/g for Ag, Cu, and Zn, respectively), the metal content achieved in HCR at a molar ratio of aluminum atoms to biocide metal atoms of 1:3 corresponds to the degree of substitution of 56, 83 and 46% for silver, copper and zinc, respectively (samples AgHCR, CuHCR$_1$ and ZnHCR$_1$). In solution hydrated cations of copper and zinc have the same regular configuration M(H$_2$O)$_6^{2+}$, size and diffusion coefficients [42], but in conditions of “solid” ion exchange the incorporation of zinc ions into the clinoptilolite structure encountered difficulties.

*Table 1*

**Conditions for the preparation of modified forms of HCR and their chemical composition**

<table>
<thead>
<tr>
<th>Sample</th>
<th>HCR</th>
<th>AgHCR</th>
<th>CuHCR$_1$</th>
<th>CuHCR$_2$</th>
<th>ZnHCR$_1$</th>
<th>ZnHCR$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aluminum : biocide metal</td>
<td>1:3</td>
<td>1:3</td>
<td>1:5.2</td>
<td>1:3</td>
<td>1:5.2</td>
<td></td>
</tr>
<tr>
<td>Weight ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zeolite:salt</td>
<td>1:1.5</td>
<td>1:1.5</td>
<td>1:2.55</td>
<td>1:1.2</td>
<td>1:2</td>
<td></td>
</tr>
<tr>
<td>Yield, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>92</td>
<td>88</td>
<td>95</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Si/Al</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>3.6</td>
<td>3.9</td>
<td>4.1</td>
<td>3.65</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Cation content per Al atom</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>0.25</td>
<td>0.11</td>
<td>0.08</td>
<td>0.03</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>K</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Ca</td>
<td>0.19</td>
<td>0.10</td>
<td>0.03</td>
<td>0.02</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Mg</td>
<td>0.15</td>
<td>0.09</td>
<td>0.04</td>
<td>0.02</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Ag</td>
<td></td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td></td>
<td></td>
<td>0.36</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Biocide metal content, mg/g</td>
<td>130</td>
<td>63</td>
<td>72</td>
<td>30</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>mmol/g</td>
<td>1.2</td>
<td>1.0</td>
<td>1.14</td>
<td>0.46</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Scientific ion exchange capacity, meq/v/g</td>
<td>3.03</td>
<td>3.03</td>
<td>2.87</td>
<td>2.78</td>
<td>3.00</td>
<td>2.87</td>
</tr>
</tbody>
</table>
Nevertheless, the achieved metal content in HCR is significantly higher than that reported for the clinoptilolite-containing tuff from the Semnan deposit, Iran (0.24, 0.28, and 0.24 mmol/g of Ag, Cu, and Zn, respectively [14]); the silver content in AgHCR is one and a half times higher than in natural clinoptilolite (0.84 mmol/g [28]) used for the removal of bacteria *Escherichia coli* and heavy metals from aqueous solutions; the content of copper and zinc even for CuHCR and ZnHCR is two times higher than in natural clinoptilolite from the sedimentary deposit Zlatokop, Serbia (0.41 and 0.225 mmol/g of Cu and Zn, respectively [26]), used for disinfection of secondary effluent water.

An increase in the amount of the reacting salt leads to an increase in the degree of copper substitution (sample CuHCR contains 1.14 mmol/g, 95% substitution) and causes a significant increase in the degree of zinc substitution (sample ZnHCR, 0.89 mmol/g, 89%). At the same time, an increase in the amount of the reacting salt leads to an increase in the duration of washing and to a greater loss of small crystallites, which causes a decrease in the yield of the target product.

The greatest change in the silicate modulus Si/Al and leaching of aluminum atoms (up to 10%) occurs when copper Cu$^{2+}$ ions are introduced into the zeolite structure; when silver Ag$^{+}$ ions are introduced, the modulus does not change. Ion exchange mainly takes place with the participation of sodium Na$^{+}$ ions, relatively heavy and large potassium ions (sodium and potassium ion radii 0.098 and 0.138 nm, respectively) do not participate in ion exchange reactions.

The introduced ions of bioactive metals are evenly distributed over the surface of the zeolite, as evidenced by layered XRED images of silver (Fig. 5) and copper (Fig. 6) distribution; at a small magnification, it can be seen that silver ions repeat the locations of aluminum atoms (Fig. 5); with a higher magnification, the pattern of the arrangement of copper ions is absolutely uniform (Fig. 6).

Comparison of the powder XRD patterns of the initial and modified samples (Fig. 7) confirms the retention of the crystal structure of the heulandite-clinoptilolite during ion-exchange reactions.

With the exception of the $v_{asym}$ vibration band, no notable changes were observed in the FTIR spectra of the modified samples in comparison with the
vibration bands of the initial zeolite. For the modified samples, the band of external asymmetric stretching vibrations narrows and has one maximum \(- v_{\text{asym}} = 1056 \text{ cm}^{-1}\) for AgHCR and CuHCR, and 1035 cm\(^{-1}\) for ZnHCR, although the value of the silicate modulus Si/Al for ZnHCR\(_1\) changes insignificantly.

Fig. 5. SEM image of AgHCR and layered XRED images of aluminum (Al) and silver (Ag) distribution

Fig. 6. SEM image of CuHCR\(_1\) and layered XRED images of aluminum (Al) and copper (Cu) distribution

Fig. 7. Powder X-ray diffraction patterns of the HCR and its modified forms enriched with silver (AgHCR), copper (CuHCR) and zinc (ZnHCR)
In one of the early studies [29], it was noted that the ratio of the absorbance of asymmetric stretching vibration of the external tetrahedra with frequency $\nu_{\text{asym}}$ to the absorbance of internal bending vibration with frequency $\delta$ for natural Mexican zeolite is 1.34 and increases to 1.64 when clinoptilolite-heulandite is enriched with silver ions. For the initial zeolite HCR, the ratio absorbance ($\nu_{\text{asym}}$) : absorbance ($\delta$) is 1.3; upon the introduction of copper ions, it increases to 1.4, and upon the introduction of silver and copper ions, to 1.8.

A narrow absorption band at 1385 cm$^{-1}$ typical for NO stretching vibrations in nitro compounds was observed in IR spectra of insufficiently washed silver-enriched HCR, this effect can be used to monitor the purity of silver-containing samples.

The introduction of ions of bioactive metals into the crystal structure changed the adsorption characteristics of heulandite. The results of measurements carried out under static conditions at room temperature are shown in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HCR</th>
<th>AgHCR</th>
<th>CuHCR$_1$</th>
<th>CuHCR$_2$</th>
<th>ZnHCR$_1$</th>
<th>ZnHCR$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water adsorption, mmol/g at p/p$_0$=0.4</td>
<td>4.75</td>
<td>4.0</td>
<td>5.4</td>
<td>5.6</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6.4</td>
<td>7.2</td>
<td>7.9</td>
<td>7.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Benzene adsorption, mmol/g at p/p$_0$=1.0</td>
<td>0.18</td>
<td>0.13</td>
<td>0.46</td>
<td>0.46</td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

The replacement of comparatively small ions of sodium (radius 1.16 Å), calcium (1.14 Å), and magnesium (0.86 Å) by larger ions of silver (1.26 Å) leads to a decrease in the adsorption capacity for both water vapor and benzene. Copper Cu$^{2+}$ and zinc Zn$^{2+}$ ions have small radii, 0.87 and 0.88 Å, respectively, and this explains the increase in the adsorption capacity for water vapor in copper- and zinc-containing samples. However, this alone cannot explain the significant increase in the adsorption of benzene, especially for the copper-containing forms, and it should be concluded that the introduction of divalent Cu$^{2+}$ and Zn$^{2+}$ ions leads to a noticeable change in the hydrophilic-hydrophobic properties of the zeolite surface.
Bacteriostatic properties

Bacteriostatic properties of zeolite samples were determined by the disk diffusion (Kirby-Bauer) method using the cultures of gram-negative bacterium Escherichia coli \((E. coli, \text{strain ATTC 8739})\), gram-positive bacteria Staphylococcus aureus \((S. aureus, \text{ATTC 6538})\) and Bacillus subtilis \((B. subtilis, \text{ATTC 6633})\), fungal pathogenic yeast Candida albicans \((C. albicans, \text{ATTC 10231})\) and a fungus Aspergillus niger \((A. niger, \text{ATTC 16404} – A. brasiliensis)\) placed \((10^9 \text{ CFU/ cm}^3)\) on Mueller–Hinton agar \((3 \text{ mm deep})\) poured into \(100 \text{ mm Petri dishes}\). Before testing the antibacterial activity, all dry zeolite products were sterilized at 70°C for 2 hours in a dry sterilizer. No microbial contamination of the prepared samples was found. 0.1 g of zeolite in the form of pellets with 8 mm in diameter was placed into the plates. The plates contaminated with \(E. coli, S. aureus\) and \(B. subtilis\) were incubated at 37°C over 5% CO\(_2\) medium and, finally, the width of inhibition zone of each sample in the plates was measured at the end of the first day. The plates contaminated with \(C. albicans\) and \(A. niger\) were incubated at 25°C during 3-4 days, results are given in the Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zeolite</th>
<th>HCR</th>
<th>AgHCR</th>
<th>CuHCR(_1)</th>
<th>CuHCR(_2)</th>
<th>ZnHCR(_1)</th>
<th>ZnHCR(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>0</td>
<td>21</td>
<td>15</td>
<td>36</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>0</td>
<td>19</td>
<td>19</td>
<td>53</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
<td>0</td>
<td>30</td>
<td>21 – 36*</td>
<td>41</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>Candida albicans</td>
<td></td>
<td>0</td>
<td>20.5</td>
<td>15</td>
<td>54</td>
<td>15*</td>
<td>61</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td></td>
<td>0</td>
<td>25</td>
<td>14*</td>
<td>65</td>
<td>17</td>
<td>69</td>
</tr>
</tbody>
</table>

*secondary growth

Prepared silver-containing heulandite-clinoptilolite shows bacteriostatic activity against all microorganisms used; sample CuHCR\(_1\) with a relatively low copper content is inferior to it in activity, and sample CuHCR\(_2\) with an increased copper content shows significantly higher activity, especially against staphylococcus and fungi. The zinc-containing form ZnHCR\(_1\) is inactive against \(E. coli\) and staphylococcus, but has weak bacteriostatic activity against other
microorganisms, an increase in the amount of bioactive metal makes sample ZnHCR\textsubscript{2} the most active against all applied microorganisms.

However, the picture of bacteriostatic activity, especially with the participation of the zinc-containing form, changes dramatically in the case of using mechanical mixtures of metal-containing heulandites, and this is shown in Figures 8-10.

![Graph](image)

**Fig. 8.** Inhibition zones of *E. coli* growth by AgHCR, CuHCR\textsubscript{1}, ZnHEU\textsubscript{1} and their mixtures in different molar ratios

Despite the fact that the zinc form ZnHCR\textsubscript{1} is inactive to *E. coli*, even a small addition of the silver AgHCR or copper CuHCR\textsubscript{1} form dramatically increases the activity; the highest bacteriostatic activity is observed for the $\frac{1}{4}$ZnHCR + $\frac{3}{4}$CuHCR mixture (Fig. 8). The synergistic effect is also manifested in relation to the both Gram positive bacteria (Fig. 9), the mixtures $\frac{1}{4}$AgHCR+$\frac{3}{4}$CuHCR, $\frac{1}{2}$AgHCR+$\frac{1}{2}$ZnHCR and $\frac{1}{4}$CuHCR+$\frac{3}{4}$ZnHCR have the greatest activity against staphylococcus, and mixtures of copper and zinc forms are most effective against hay bacillus.

![Graph](image)

**Fig. 9.** Inhibition of Gram positive bacteria by AgHCR, CuHCR\textsubscript{1}, ZnHEU\textsubscript{1} and their mixtures in different molar ratios
In inhibiting the growth of fungi (Fig. 10), mixtures with the AgHCR show low activity, the most effective are mixtures of CuHCR and ZnHCR, which is very important from a practical point of view, to replace expensive silver (approx. 855 $/kg, July 2021) with cheaper copper (10 $/kg) and zinc (3 $/kg).

As follows from the data obtained, a mixture with a high copper content, Cu/Zn = 3, is the most effective against *E. coli* and hay bacillus, while the zone of inhibition is two times higher than that of the silver-containing form. On the contrary, against staphylococcus and *C. albicans*, the most effective is a mixture with a high zinc content, Zn/Cu = 3, and the maximum inhibition of the growth of *A. niger* is caused by a mixture with equal amounts of copper and zinc.

**Table 3**

Relative measure of the synergistic action of CuHCR₁ and ZnHEU₁ against the microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Weight ratio CuHCR₁/ZnHCR₁</th>
<th>(\frac{1}{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.67</td>
<td>2.4</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>1.25</td>
<td>1.21</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1.74</td>
<td>1.37</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1.5</td>
<td>1.57</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>3.1</td>
<td>3.33</td>
</tr>
</tbody>
</table>

Table 3 shows the values of the ratio of the diameter of the inhibition zone by the mixture (\(D_{CuZn}\)) to the total diameter of the zones of inhibition by individual
copper- and zinc-containing zeolites (D_{Cu} and D_{Zn}, respectively), D_{CuZn}/(D_{Cu}+D_{Zn}), as relative measures of the synergistic action of copper and zinc against the microorganisms used in the study.

A similar synergistic effect was noted in the study of synthetic zeolite fibres [21]. It has been shown that the bacterial inhibition for nanofibres containing a three metal (Ag+Cu+Zn) nano-zeolite Y is higher than for containing bimetals (Ag+Cu, Ag+Zn, or Cu+Xn), while for containing monometals (Ag, Cu, or Zn), it was lower compared to the others.

The obtained bactericidal adsorbents can be used as fillers in the production of packaging paper to impart bacteriostatic properties to its surface, and the results of research in this direction are presented in the second part of this work.

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References:


