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BACTERICIDAL METAL-CONTAINING ZEOLITES

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The coronavirus pandemic has increased interest in antibacterial agents containing bioactive metals, for which zeolites are promising carriers. On the other hand, zeolite adsorbents and ion exchangers containing bioactive metals and endowed with bactericidal properties are promising for water treatment and other environmental and medical applications. Silver-, copper-, and zinc-containing microporous materials have been prepared on the base of natural analcime, phillipsite and heulandite from Georgian manifestations using ion-exchange reactions between zeolite microcrystals and a salt of a corresponding transition metal in the solid phase followed by washing with distilled water. Synthesized in such way adsorbent-ion-exchangers are characterized by chemical composition based on the X-ray energy dispersion spectra, powder X-ray diffraction patterns, Fourier transform infra-red spectra and low-temperature adsorption-desorption isotherms of N_2*. Obtained materials remain the zeolite crystal structure and contain 130–230 mg/g of silver, 65–72 mg/g of copper, and 58–86 mg/g of zinc, as compared with modified samples of synthetic type A zeolite containing up to 380 mg/g of silver, 150 mg/g of copper, and 150 mg/g of zinc. Prepared metal-containing materials show bacteriostatic activity against Gram negative bacterium Escherichia coli, Gram positive bacteria Staphylococcus aureus and Bacillus subtilis, fungal pathogenic yeast Candida albicans, and a fungus Aspergilus niger, and natural zeolites enriched with biometals exhibit a synergistic effect – their mixtures have a higher bacteriostatic activity. It is shown that mixtures of copper and zinc forms have a higher activity than the silver-containing form, which is very important from a practical point of view for replacing expensive silver with cheaper copper and zinc. It has been found that the bacteriostatic activity of metal-containing zeolites is determined not only and not so much by the ions of bioactive metals released into the liquid medium, but an important role in inhibiting the growth of microorganisms plays a type of zeolite matrix. Despite the relatively low ion-exchange capacity, heulandite turned out to be a fairly effective matrix for bioactive metals.*

Keywords: *silver-, copper-, zinc-containing analcime, phillipsite and heulandite, bacteriostatic activity*

INTRODUCTION

Synthetic zeolites (aluminosilicates with the general formula $M_nSi_xAl_nO_{2(n+x)}{\cdot}mH_2O$, where exchangeable M^{+n} ions are compensating the negative charge of the crystal lattice constructed from alternating SiO_4 and AlO_4^- tetrahedra) in which ions of alkali (Na^+, K^+) or alkali-earth (½Ca^{2+}, ½Mg^{2+}) metals M are partially replaced by silver ions Ag^+ have been used as bactericidal materials since the end of the last century [1], and subsequently synthetic and natural zeolites containing such bioactive metals as copper and zinc were obtained and studied; a review of published work in this area is given in our recent publication on bactericidal adsorbents obtained by ion-exchange modification of natural phillipsite [2].

In the context of the coronavirus pandemic, not only the demand for various disinfectants has increased, but also the interest in obtaining new antibacterial and antiviral materials, among which metal-containing zeolites (MZs, Me = Ag, ½Cu, ½Zn, *etc.*) are recognized as very promising [3], especially in antibiotic-free strategies [4]. It is generally accepted that silver ions and nanoparticles have the highest activity against many microorganisms, but the practical application of AgZs is limited due to the high cost of silver (640 $/kg, August 2022); zinc is a fairly cheap metal (approx. 3.4 $/kg), but published data indicate a low activity of ZnZs [2, 5–11], copper meets the criteria of economy (7.6 $/kg) and efficiency of CuZs [2, 5–12].

It is believed that zeolites that do not contain transition metals are not active against microorganisms [6, 13], and the porous zeolite structure of MZs is only a depot for bioactive metal ions [14], but recently it was found that in some cases, antibacterial activity can be associated with the zeolite matrix itself [9, 11].

The aim of our study was to enrich natural Georgian zeolites such as analcime (crystal chemical data $Na_{16}(H_2O)_{16}[Al_{16}Si_{32}O_{96}]$-**ANA**, scientific weight capacity SWC = 5.1 meq/g), phillipsite (|K_2 (Ca,Na_2)$_2$ (H_2O)$_{12}$| [$Al_6Si_{10}O_{32}$]-**PHI**, SWC = 3.23 meq/g) and heulandite-clinoptilolite (|$Ca_4(H_2O)_{24}$|[$Al_8Si_{28}O_{72}$]-**HEU**, SWC = 3.08 meq/g) with silver, copper and zinc, and to study their bacteriostatic activity against a wide range of microorganisms (bacteria, yeast, fungus); synthetic type A zeolite (|$Na_{12}(H_2O)_{27}$|$_8$[$Al_{12}Si_{12}O_{48}$]$_8$-**LTA**, SWC = 6.23meq/g) was used for comparison.

EXPERIMENTAL

Materials. Preparation of MZs by “ion exchange synthesis” was carried out using Georgian natural analcime from the Chachubeti manifestation (Eastern Georgia) described in [15], phillipsite-containing tuff rock from Shukhuti (Western Georgia) described in [2], and heulandite from the Rkoni plot of Tedzami-Dzegvi deposit (Eastern Georgia, H_R) described in [16], as well as using synthetic type A zeolite prepared by hydrothermal recrystallization of natural analcime [17].

The analcime-containing rock was crushed in the planetary micro mill Pulverisette 7 (Fritsch Laboratory Instruments, Idar-Oberstein, Germany) to a size less than 0.063 mm (240 BSS mesh), used without washing, and named as A_{CH} (analcime from Chachubeti). Crushed and sieved phillipsite- and heulandite-containing rocks were washed by diluted HCl solution (0.025 N) to remove clay and other impurities, and named as P_{SH} (phillipsite from Shukhuti) and H_R (heulandite from Rkoni), respectively. As-synthesized type A zeolite crystallites 3 to 5 µm in size were used in experiments.

Analytical grade silver nitrate $AgNO_3$, copper chloride $CuCl_2$, and zinc chloride $ZnCl_2$ were purchased from Merck KGaA (Darmstadt, Germany) and used without any further purification.

Preparation of MZs. Ion exchange was carried out as follows: powder of zeolite and the corresponding salt were mixed in weight ratio 1:6 and thoroughly grinded in an agate mortar for 10 minutes for $AgNO_3$-containing mixtures and for 15 min for $CuCl_2$- and $ZnCl_2$-containing mixtures. The solid mixture was then transferred to a filter and washed with distilled water until the absence 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 with a maximum content of corresponding bioactive metal are labeled as AgA, AgA_{CH}, AgP_{SH}, AgH_R (silver-containing type A zeolite, analcime, phillipsite and heulandite, respectively), CuA, CuA_{CH}, CuP_{SH}, CuH_R (copper-containing zeolites), and ZnA, ZnA_{CH}, ZnP_{SH}, ZnH_R (zinc-containing zeolites).

Characterization of samples. Chemical composition of raw material and prepared samples was determined by elemental analyses carried out using atomic absorption spectrometer (model 300, Perkin-Elmer, UK) and energy dispersive X-ray (EDS) analysis. The crystalline phase was identified by powder X-ray diffraction (XRD) patterns obtained from a modernized Dron-4 X-ray diffractometer employing the CuK_α line (λ = 0.154056 nm). The samples were scanned in the 2Θ range of 5 to 50° with a 0.02° step at a scanning speed of 1°/min. Fourier transform infrared spectra were collected by a 10.4.2 FTIR spectrometer (Perkin-Elmer, UK) over the range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1} using the KBr pellet technique for sample preparation. The surface morphology of the samples was observed by a scanning electron microscope JSM6510LV (Jeol, Japan) equipped with a X-Max 20 analyzer (Oxford Instruments, UK) for EDS. Nitrogen adsorption-desorption isotherms were measured at 77 K using an ASAP 2020 Plus physisorption analyzer (Micromeritics, Norcross, GA, USA), after evacuation of the samples at 350 °C during 2 hours; water adsorption capacity was measured under static conditions.

Metal release and antibacterial activity. The determination of the amount of metals released from MZs in normal salina solution (9 g of NaCl in 1 L of deionized water) was carried out under static conditions in a thermostatic bath (Grant Instruments OLS26 Aqua Pro) at a temperature of 37±0.1 °C, without stirring or shaking. Sampling for analysis was carried out after 1, 3, 6 and 24 h, as well as after 48 and 72 h for copper- and zinc-containing samples after loading 0.1 gram of zeolite in 100 ml of salina. Analysis was carried out using an ion chromatograph CDD-10Avp/10ASP (Shimadzu, Japan).

Bacteriostatic properties of zeolite samples were determined by the disk diffusion (Kirby-Bauer) method in standard conditions using the cultures of Gram negative bacterium *Escherichia coli* (strain ATTC 8739), Gram-positive bacteria *Staphylococcus aureus* (ATTC 6538) and *Bacillus subtilis* (ATTC 6633), fungal pathogenic yeast *Candida albicans* (ATTC10231) and a fungus *Aspergillus niger* (ATTC16404 –

A. brasiliensis) placed (109 CFU/cm^3) on Mueller–Hinton agar (3 mm deep) poured into 100 mm Petri dishes. Before testing the antibacterial activity, all dry zeolite products were sterilized at 70 °C for 2 h in a dry sterilizer. No microbial contamination of the prepared samples was found.0.2 g of zeolite in the form of pellets with 8 mm in diameter was placed into the plates. The plates contaminated with *E. coli, St. aureu*s 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 *Candida albicans* and *Aspergillus niger* were incubated at 25 °C during 3–4 days. All experiments were done in triplicate and the values obtained were averaged to give the final data with standard deviations of 3–7 %.

RESULTS AND DISCUSSION

Chemical composition and crystal structure. Chemical composition of native zeolites and their modified forms with a maximum silver, copper or zinc content are listed in the Table 1 in terms of the empirical formulas $(M_aNa_bK_cCa_dMg_eMe_f)[Al_xSi_yO_{32}]{\cdot}nH_2O$, where z = 96 for analcimes, z = 32 for phillipsites, z = 72 for heulandites, and z = 48 for type A zeolites, Me^+ ion corresponds to the impurity metals ($^1/_3Fe^{3+}$, $½Cu^{2+}$, $½Zn^{2+}$, $½Mn^{2+}$, *etc.*), $M^+ = Ag^+$, $½Cu^{2+}$, or $½Zn^{2+}$, and deviations are given in parentheses.

Table 2. Chemical composition of native and modified zeolites

Sample	Empirical formula	Si/Al
A_{CH}	$(Na_{9.25}K_{2.25}Ca_{1.1}Mg_{1.0})Me_{0.50}[Al_{16.2}Si_{32.0}O_{96}]{\cdot}18.4H_2O$	1.98(15)
AgA_{CH}	$Ag_{9.5}(Na_{1.9}K_{1.7}Ca_{0.5}Mg_{0.5})Me_{0.10}[Al_{15.2}Si_{32.0}O_{96}]{\cdot}18.9H_2O$	2.10(20)
CuA_{CH}	$Cu_{3.7}(Na_{3.2}K_{1.3}Ca_{0.75}Mg_{0.75})Me_{0.20}[Al_{15.0}Si_{32.0}O_{96}]{\cdot}19.5H_2O$	2.13(15)
ZnA_{CH}	$Zn_{3.8}(Na_{3.7}K_{1.4}Ca_{0.75}Mg_{0.75})Me_{0.20}[Al_{15.1}Si_{32.0}O_{96}]{\cdot}19.2H_2O$	2.13(15)
P_{SH}	$(Na_{1.30}K_{2.0}Ca_{0.30}Mg_{0.25})Me_{0.10}[Al_{4.50}Si_{11.80}O_{32}]{\cdot}11.4H_2O$	2.62(16)
AgP_{SH}	$Ag_{3.42}(Na_{0.01}K_{0.08}Ca_{0.25}Mg_{0.20})Me_{0.03}[Al_{4.44}Si_{11.20}O_{32}]{\cdot}14.2H_2O$	2.52(15)
CuP_{SH}	$Cu_{1.80}(Na_{0.22}K_{0.17}Ca_{0.125}Mg_{0.12})Me_{0.075}[Al_{4.50}Si_{11.70}O_{32}]{\cdot}14.3H_2O$	2.60(16)
ZnP_{SH}	$Zn_{1.84}(Na_{0.01}K_{0.35}Ca_{0.075}Mg_{0.125})Me_{0.025}[Al_{4.47}Si_{11.70}O_{32}]{\cdot}15.6H_2O$	2.64(16)
H_R	$Na_{1.95(10)}K_{0.47(2)}Ca_{1.48(7)}Mg_{1.16(6)}Me_{0.13(1)}(Al_{7.83(48)}Si_{28.2(1.7)}O_{72}){\cdot}24.0H_2O$	3.6(2)
AgH_R	$Ag_{3.6(2)}Na_{0.87(4)}K_{0.47(2)}Ca_{0.79(4)}Mg_{0.71(4)}(Al_{7.9(5)}Si_{28.4(1.7)}O_{72}){\cdot}24.1H_2O$	3.6(2)
CuH_R	$Cu_{3.15(15)}Na_{0.23(1)}K_{0.38(2)}Ca_{0.15(1)}Mg_{0.15(1)}(Al_{7.5(5)}Si_{29.2(1.8)}O_{72}){\cdot}24.4H_2O$	3.9(2)
ZnH_R	$Zn_{2.50(12)}Na_{0.55(3)}K_{0.40(2)}Ca_{0.40(2)}Mg_{0.55(3)}(Al_{7.8(5)}Si_{28.8(1.8)}O_{72}){\cdot}24.7H_2O$	3.7(2)
A	$Na_{11.25(25)}(K,½Ca,½Mg)_{0.7(1)}(Al_{11.95(25)}Si_{12.3(3)}O_{48}){\cdot}18.0H_2O$	1.03(5)
AgA	$Ag_{10.3(4)}Na_{1.2(5)}(K,½Ca,½Mg)_{0.20(5)}(Al_{11.7(3)}Si_{12.3(3)}O_{48}){\cdot}18.6H_2O$	1.05(6)
CuA	$Cu_{5.0(3)}Na_{1.35(25)}(K,½Ca,½Mg)_{0.35(7)}(Al_{11.7(3)}Si_{12.3(3)}O_{48}){\cdot}19.4H_2O$	1.05(6)
ZnA	$Zn_{5.05(25)}Na_{1.2(3)}(K,½Ca,½Mg)_{0.20(5)}(Al_{11.5(3)}Si_{12.2(3)}O_{48}){\cdot}19.7H_2O$	1.06(6)

According to the elemental analysis data, when silver, copper, and zinc ions are introduced into the crystal lattice of zeolites, the ratio Si/Al changes insignificantly, and the number of water molecules in the crystal lattice increases.

Ion exchange reactions do not change the crystal structure of the zeolite, this is confirmed by the powder X-ray diffraction patterns of the modified samples (details for modified phillipsites, analcimes and heulandites see in [2], [11, 15] and [16], respectively; characteristic peaks of the PHI, ANA and HEU structures remain in XRD patterns, only their intensities change; XRD pattern of the LTA structure [17] remains unchanged). No notable changes were observed in the IR spectra of the modified samples as compared with the vibration bands of starting zeolitic material, only the intensity of the broad band at 3200–3700 cm^{-1} corresponding to the asymmetric stretching of OH group is increased due to the larger number of water molecules in the samples containing silver, copper, and zinc.

The degree of substitution (the ratio of the charge of transition metal ions to the number of aluminum atoms, DS = a/x for AgZs, and DS = 2a/x for CuZs and ZnZs) is highest for synthetic zeolite and lowest for heulandite and analcime, but the specific content of bioactive metals in analcimes is not inferior to their content in phillipsites (see Table 2).

The degree of substitution and the content of metals in heulandite from the Rkoni plot is somewhat lower, but comparable with the maximum possible content of silver, copper, and zinc calculated from ion-exchange isotherms measured on natural clinoptilolite from Gördes, Turkey (1.85, 1.21 and 1.03 mmol/g for Ag, Cu and Zn, respectively) [5]. In [2], results for phillipsite were compared with the literature data for different clinoptilolites [5, 6, 9, 18], and it was shown that phillipsite is a more promising carrier of silver, copper, and zinc than natural clinoptilolite. The DS achieved for synthetic zeolite A corresponds to silver content of 3.54, copper content of 2.33, and zinc content of 2.36 mmol/g, that are much higher than those shown in [6] – 0.27, 0.27, and 0.28 mmol/g, respectively.

Table 2. Degree of substitution and metal content in modified zeolites

Sample	Degree of substitution	Metal content	
		mg/g	mmol/g
AgA$_{\mathrm{CH}}$	0.625	234	2.17
CuA$_{\mathrm{CH}}$	0.49	65	1.03
ZnA$_{\mathrm{CH}}$	0.50	69	1.05
AgP$_{\mathrm{SH}}$	0.77	230	2.13
CuP$_{\mathrm{SH}}$	0.80	66	1.04
ZnP$_{\mathrm{SH}}$	0.82	86	1.31
AgH$_{\mathrm{R}}$	0.46	134	1.24
CuH$_{\mathrm{R}}$	0.32	72	1.14
ZnH$_{\mathrm{R}}$	0.72	58	0.89
AgA	0.88	382	3.54
CuA	0.85	149	2.33
ZnA	0.88	154	2.36

The features of the incorporation of silver, copper, and zinc ions into the structure of the studied zeolites are discussed in our publications [2, 10, 11, 15, 16], which also show that ion exchange reactions do not significantly affect the mesopore system present in natural zeolites and their adsorption capacities. With regard to morphology, ion exchange affects the size distribution of crystallites only for phillipsites [2].

Release of metal ions. Data on the leaching of metals from modified zeolites are given in Table 3 in terms of the values of minimal inhibitory concentration (MIC) in relation to *E. coli* for the corresponding bioactive ion: MIC of silver ions toward *E. coli* is 3.996 mg Ag$^+$ in dm^3 [19] or 0.037 mM, MIC value for copper and zinc ions toward *E. coli* is 1 mM [20].

Table 3. The leaching of metals (mM/MIC) from MZs

Sample	Ion	In 1 h	In 3 h	In 6 h	In 24 h	In 48 h	In 72 h
MA$_{\mathrm{CH}}$	Ag$^+$	0.20(2)	0.43(5)	0.92(8)	1.6(2)		
	Cu^{2+}	<0.05	0.20(2)	0.34(4)	0.52(5)	0.53(5)	0.54(6)
	Zn^{2+}	<0.05	0.23(3)	0.38(5)	0.60(7)	0.61(6)	0.62(7)
MP$_{\mathrm{SH}}$	Ag$^+$	0.75(8)	1.3(2)	1.8(4)	5.2(5)		
	Cu^{2+}	<0.05	0.13(2)	0.25(3)	0.45(5)	0.46(5)	0.48(5)
	Zn^{2+}	0.08(1)	0.20(2)	0.35(4)	0.50(5)	0.52(6)	0.54(6)
MH$_{\mathrm{R}}$	Ag$^+$	0.70(8)	1.2(2)	1.5(2)	2.8(6)		
	Cu^{2+}	<0.05	0.13(2)	0.25(3)	0.45(5)	0.47(5)	0.48(5)
	Zn^{2+}	0.08(1)	0.20(2)	0.35(4)	0.50(5)	0.53(6)	0.55(6)
MA	Ag$^+$	1.05(15)	2.4(3)	3.2(6)	5.0(9)		
	Cu^{2+}	<0.05	0.11(1)	0.22(3)	0.32(4)	0.35(4)	0.36(4)
	Zn^{2+}	<0.05	<0.05	<0.05	0.15(3)	0.16(3)	0.16(3)

The amount of silver ions released from silver-enriched synthetic zeolite AgA after 1 h is somewhat higher than the MIC, from AgP_{SH} and AgH_R inhibitory concentration is reached after about 2 h, and from AgA_{CH} only after approx. 12 h. The leached amounts of copper and zinc ions from the zeolites are lower than MIC and negligible for ZnA, and this is in full agreement with the results of [9]. Type A zeolite was designed for water softening by removing divalent ions during ion-exchange reactions such as $2Na^+ \leftrightarrow Ca^{2+}(Mg^{2+})$. Apparently, type A zeolite irreversibly captures zinc ions, but copper ions retain the possibility of ion exchange with the aqueous environment. It is important to notice that the amount of the leached Cu^{2+} and Zn^{2+} ions is in the range of the maximum allowable concentrations in drinking water [21].

Bacteriostatic activity. Results of the Kirby-Bauer test are given in Tables 3–7. All original zeolites that do not contain bioactive metals were inactive, enriched synthetic zeolites equally inhibit the growth of gram-negative and gram-positive bacteria, but the copper form is not active against yeast and fungus, while the zinc form is not inferior in activity to silver, and in relation to *E. coli* even shows a slightly higher activity (Table 4). Previously, researchers from Serbia and Croatia showed that the activity of synthetic type A zeolites against *E. coli* decreases in the series AgA > CuA >> ZnA, but this discrepancy can be attributed to the use of commercial zeolite A in [9] and low degree of enrichment, about 0.25 mmol/g.

Table 4. Diameter (mm) of the zone of inhibition of the growth of microorganisms by type A zeolites

Microorganism	A	AgA	CuA	ZnA
Escherichia coli	0	23	17	26
Staphylococcus aureus	0	25	25	23
Bacillus subtilis	0	26	23	25
Candida albicans	0	20	0	24
Aspergillusniger	0	24	0	24

Of the enriched forms of analcime (see Table 5), only the silver form AgA_{CH} slows down the growth of all microorganisms used in the study, the copper form CuA_{CH} is not active at all, and the zinc form ZnA_{CH} is active only against *B. subtilis* hay bacillus. However, a mixture of silver and zinc forms in a 1:1 molar ratio shows a synergistic effect – increased activity against all microorganisms, a mixture of silver and copper forms in the same 1:1 molar ratio shows a rather high activity. A mixture of copper- and zinc-containing analcimes, which are absolutely inactive against staphylococci, shows little activity against this bacterium; the addition of inactive CuA_{CH} to ZnA_{CH} slightly increases the activity of the latter against *B. subtilis*.

Table 5. Diameter (mm) of the zone of inhibition of the growth of microorganisms by analcimes

Microorganism	A_{CH}	AgA_{CH}	CuA_{CH}	ZnA_{CH}	$(Ag+Cu)A_{CH}$	$(Ag+Zn)A_{CH}$	$(Zn+Cu)A_{CH}$
Escherichia coli	0	21	0	0	25	28	0
Staphylococcus aureus	0	25	0	0	24	27	13
Bacillus subtilis	0	23	0	20	18	29	21
Candida albicans	0	20	0	0	16	24	0
Aspergillus niger	0	25	0	0	17	35	0

Synergism was also found for enriched forms of phillipsite – the greatest activity against *E. coli* and staphylococcus is shown by a mixture (1:1) of silver and copper forms, against a black fungus – by a mixture (1:1) of silver and zinc forms (Table 6). On the other hand, mixtures of copper and zinc forms display an antagonistic effect in relation to *E. coli* (no inhibition), and the addition of inactive CuP_{SH} to ZnP_{SH} significantly decreases the activity of the latter against fungi.

To the greatest extent, the synergistic effect is manifested in enriched forms of heulandite – despite the relatively low activity of copper and zinc forms, their mixture exhibits the highest activity against all microorganisms (see Table 7, a more detailed picture of the synergistic effect for heulandites is presented in Figs. 1–3).

Table 6. Diameter (mm) of the zone of inhibition of the growth of microorganisms by phillipsites

Microorganism	P_{SH}	AgP_{SH}	CuP_{SH}	ZnP_{SH}	$(Ag+Cu)P_{SH}$	$(Ag+Zn)P_{SH}$	$(Zn+Cu)P_{SH}$
Escherichia coli	0	20	11	15	30	20	0
Staphylococcus aureus	0	19	20	0	22	16	20
Bacillus subtilis	0	16	23	22	20	19	19
Candida albicans	0	29	0	18	14	26	24
Aspergillus niger	0	27	0	28	0	29	7

Table 7. Diameter (mm) of the zone of inhibition of the growth of microorganisms by heulandites

Microorganism	H_R	AgH_R	CuH_R	ZnH_R	$(Ag+Cu)H_R$	$(Ag+Zn)H_R$	$(Zn+Cu)H_R$
Escherichia coli	0	21	15	0	27	27	40
Staphylococcus aureus	0	19	19	0	41	40	40
Bacillus subtilis	0	30	21|36*	19	40	34	50
Candida albicans	0	20.5	15	15*	40	40	50
Aspergillus niger	0	25	14*	17	44	40	70

* secondary growth

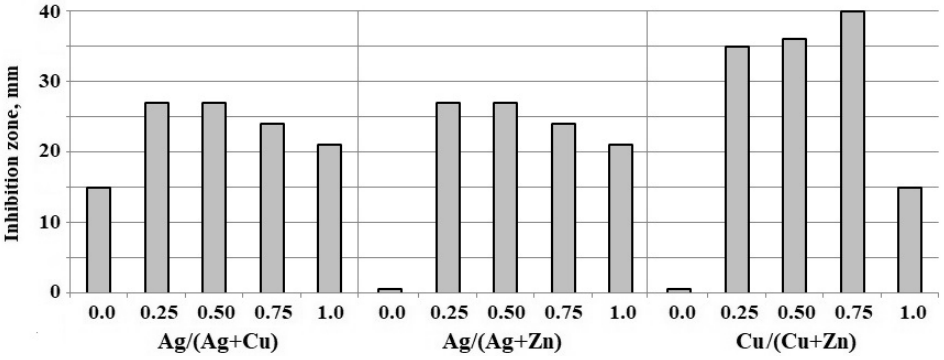

Fig. 1. Inhibition zones of *E. coli* growth by AgH_R, CuH_R, ZnH_R and their mixtures in different molar ratios

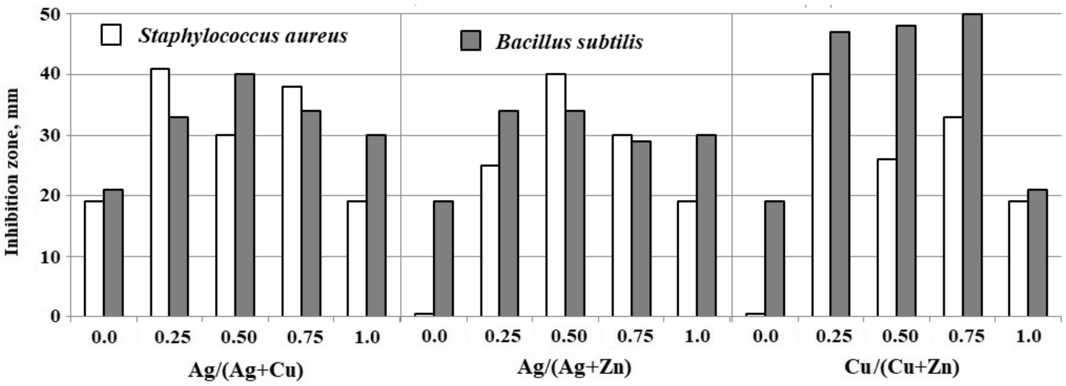

Fig. 2. Inhibition zones of Gram positive bacteria growth by AgH_R, CuH_R, ZnH_R and their mixtures in different molar ratios

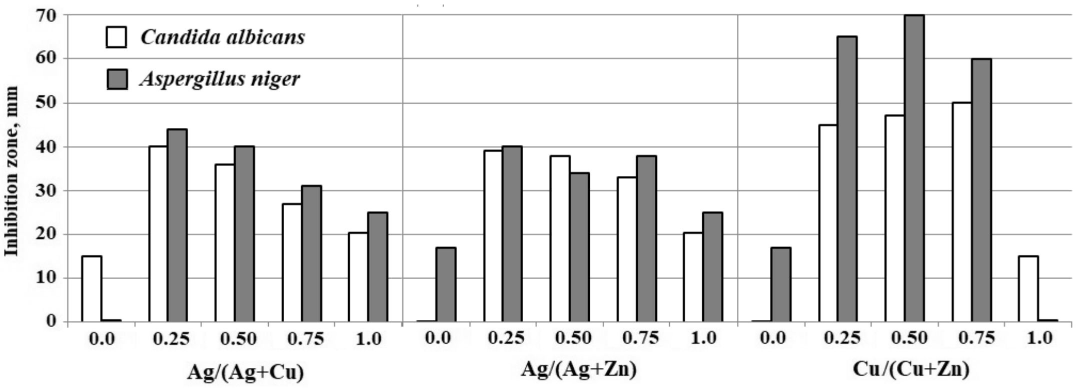

Fig. 3. Inhibition zones of fungi growth by AgH_R, CuH_R, ZnH_R and their mixtures in different molar ratios

Despite the fact that the zinc form ZnH_R is inactive to *E. coli*, even a small addition of the silver AgH_R or copper CuH_R form dramatically increases the activity; the highest bacteriostatic activity is observed for the ¼ZnH_R + ¾CuH_R mixture (Fig. 1).

The synergistic effect is also manifested in relation to the both Gram positive bacteria (Fig. 2), the mixtures ¼AgH_R + ¾CuH_R, ½AgH_R + ½ZnH_R and ¼CuH_R + ¾ZnH_R have the greatest activity against staphylococcus, and mixtures of copper and zinc forms are most effective against hay bacillus *B. subtilis*.

In inhibiting the growth of fungi (Fig. 3), mixtures with the AgH_R show low activity, the most effective are mixtures of CuH_R and ZnH_R, which is very important from a practical point of view, to replace expensive silver with cheaper copper and zinc.

As follows from the data obtained for mixtures of modified heulandites, 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.

A similar synergistic effect was noted in the study of synthetic zeolite fibres [22]. 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+Zn), while for containing monometals (Ag, Cu, or Zn), it was lower compared to the others.

Metal-containing forms of synthetic zeolite A do not exhibit synergism – mixtures demonstrate bacterial growth inhibition zones of the same size as AgA, CuA and ZnA (zone diameters from 18 to 26 mm), in the presence of a copper form in the mixture, the activity against yeast and fungus slightly decreases (zone diameters less than 20 mm).

A recent study of mono- and bimetallic antibacterial type A zeolite materials [23] showed that bimetallic samples (Ag^+ and either Cu^{2+}, Zn^{2+}) exhibit a synergetic or antagonist effects against *E. coli*, depending on the quantity of the second metal. However, this results were obtained on sequentially exchanged samples (NaA → AgNaA → AgCuNaA or AgZnNaA), and cannot be compared with our results obtained for mechanical mixtures of different metal forms.

CONCLUSION

As a result of the conducted research, it was found that solid-state ion-exchange reactions between Georgian natural zeolites (analcime, phillipsite, heulandite) and a salt of the corresponding transition metal (Ag, Cu, Zn) followed by washing lead to the formation of zeolite materials with a high content of silver (130–230 mg/g), copper (65–72 mg/g), and zinc (58–86 mg/g), comparable to the content achieved in a synthetic ion exchanger (382, 149 and 154 mg/g of silver, copper and zinc, respectively). The resulting materials have sorption and bactericidal properties, sufficient for their use in the purification and disinfection of water, as fillers in paper production [24], *etc*.

Natural zeolites enriched with biometals exhibit a synergistic effect – their mixtures have a higher bacteriostatic activity. Analysis of the obtained results shows that against *Escherichia* *coli* and hay bacillus, as well as against yeast and black fungus, a mixture of zinc and copper forms of heulandite is most effective, and against staphylococcus, all mixtures of silver, copper and zinc forms of heulandite in a molar ratio of 1:1 have approximately equal activity. Thus, despite the lower ion-exchange capacity than that of analcime and phillipsite, heulandite turned out to be a more effective matrix for bioactive metals.

Taking into account the rate of leaching of bioactive metals compared to the values of the minimal inhibitory concentrations, it can concluded that the antibacterial activity of copper- and zinc-containing zeolites could be ascribed to the corresponding zeolite matrix it self, and not only to the leached metal ions.

Thus, contrary to the opinion that the bacteriostatic activity of metal-containing zeolites is determined exclusively by the ions of bioactive metals released into the liquid medium, the study has shown that the type of zeolite also plays a very important role.

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Бактерицидні металовмісні цеоліти

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Пандемія коронавірусу підвищила інтерес до антибактеріальних засобів, що містять біоактивні метали, перспективними носіями яких є цеоліти. З іншого боку, цеолітові адсорбенти та іонообмінники, що містять біоактивні метали та наділені бактерицидними властивостями, є перспективними для очищення води та інших екологічних і медичних застосувань. Срібло-, мідь- та цинковмісні мікропористі матеріали були отримані на основі природного анальциму, філіпситу та гейландиту з грузинських проявів за допомогою іонообмінних реакцій між мікрокристалами цеоліту та сіллю відповідного перехідного металу в твердій фазі з подальшим промиванням дистильованою водою. Синтезовані таким чином адсорбенти-іонообмінники характеризуються хімічним складом на основі енергетичних дисперсійних спектрів рентгенівського випромінювання, порошкових рентгенограм, інфрачервоних фур'є-спектрів та ізотерм низькотемпературної адсорбції-десорбції азоту. Отримані матеріали зберігають кристалічну структуру цеоліту і містять 130–230 мг/г срібла, 65–72 мг/г міді, 58–86 мг/г цинку в порівнянні з модифікованими зразками синтетичного цеоліту A, що містять до 290 мг/г срібла, 75 мг/г міді та 100 мг/г цинку. Приготовані металовмісні матеріали виявляють бактеріостатичну активність щодо грамнегативних бактерій Escherichia coli, грампозитивних бактерій Staphylococcus aureus і Bacillus subtilis, патогенних дріжджів Candida albicans і грибка Aspergilus niger, а природні цеоліти, збагачені біометалами, проявляють синергетичну дію – у їхньої суміші більш висока бактеріостатична активність. Показано, що суміші форм міді і цинку мають більш високу активність, ніж срібловмісна форма, що дуже важливо з практичної точки зору для заміни дорогого срібла на більш дешеві мідь і цинк. Встановлено, що бактеріостатична активність металовмісних цеолітів визначається не тільки і не стільки вивільненими в рідке середовище іонами біоактивних металів, скільки важливу роль у пригніченні росту мікроорганізмів відіграє тип цеолітної матриці. Незважаючи на відносно низьку іонообмінну здатність, гейландит виявився досить ефективною матрицею для біоактивних металів.

***Ключові слова**: срібло-, мідь-, цинк-вмісні анальцим, філіпсит та гейландит, бактеріостатична активність*

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