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SILVER-AND ZINC-CONTAINING BACTERICIDAL PHILLIPSITES

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Abstract. Silver- and zinc-containing zeolite materials have been prepared on the basis of Georgian phillipsiteusing ion-exchange reactions between zeolite and a salt of a transition metal in the solid phase followed by washing. Synthesized adsorbent-ion-exchangers are characterized by chemical analysis and water sorption data, XRD patterns, FTIR spectra, SEM images; they remain the crystal structure and general properties of phillipsite, contain up to 230 mg/g of silver and up to 86 mg/g of zinc, and show bactericidal and bacteriostatic activity towards *Escherichia coli*. The procedure of ion-exchange synthesis leads to a significant increase in the dispersion of the material. The compliance of proposed method with high environmental standards is confirmed by its low Sheldon's factor E in comparison with the similar green chemistry metrics of conventional methods of the ion exchange in solutions.

Keywords: *phillipsite, silver, zinc, ion exchange, antimicrobial activity, Escherichia coli.*

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1. Introduction

The environmental, sanitary and medical use of zeolites ($Me_nSi_xAl_nO_{2(n+x)}\cdot mH_2O$, Me = Na, K, … ½Ca, ½Mg, …) is due to the complex of their properties, among which an important place is occupied by the ability of zeolites to enter into ion exchange reactions with the participation of Me^{+n} ions compensating the negative charge of the crystal lattice constructed from SiO_4 and AlO_4^-tetrahedra.

Utilization of the natural zeolites in water treatment has increasing interest due to their availability and low cost. At first, most of the studies in this area were concentrated on the use of clinoptilolite in the removal of ammonium ions (Klieve & Semmens, 1980; Jama & Yücel, 1989; Langella *et al.*, 2000) and heavy metals (Semmens & Seyfard, 1978; Semmens & Martin, 1988; Zamzow & Murphy, 1992; Kesraoui-Ouki *et al.*, 1993; Zamzow & Schultze, 1995; Langella *et al.*, 2000).

On the other hand, started at the beginning of the 21st century and continuing to this day, studies showed that natural and synthetic zeolites exchanged by ions of silver, copper, zinc or some other transition metals (M-Z) exhibit antimicrobial activity toward a broad range of microorganisms (Kawahara *et al.*, 2000; Rivera-Garza *et al.*, 2000; Top & Ülkü, 2004; De la Rosa Gomez *et al.*, 2008; Ferreira *et al.*, 2012; Hrenovic *et al.*, 2012; Guerra *et al.*, 2012; Hrenovic *et al.*, 2013; Akhigbe *et al.*, 2014; Demirci *et al.*, 2014; Rossainz-Castro *et al.*, 2016). In comparison to other transition metal-containing zeolites, Ag-Z exhibits the most powerful antibacterial activity (Top & Ülkü, 2004; Hrenovic *et al*., 2013; Demirci *et al*., 2014). In general, silver is considered as antibacterial agent with well-known mode of action, bacterial resistance against silver is well described (Klasen, 2000), similarities and differences between silver ions and silver in nanoforms as antibacterial agents were discussed recently (Kędziora *et al*., 2018).

Several studies showed that zeolites not containing silver, copper, zinc or other transition metals (such as mercury, cadmium, chromium, and lead) are not active toward microorganisms (Hrenovic *et al*., 2012; Jiraroj *et al*., 2014). It is believed that the porous zeolite structure enables metal cations to move freely from the lattice to the environment, and this seems to be responsible for their activity toward microorganisms (Kwakye-Awuah *et al*., 2008), but it has recently been established that in some cases the antibacterial activity could be attributed to the M-Z itself (Milenkovic *et al*., 2017).

From a practical point of view, it was suggested (Demirci *et al*., 2014) that zeolite formulations could be combined with various materials used in manufacturing medical devices, surfaces, textiles, or household items where antimicrobial properties are required. Other problem that can be solved by application of metal-containing zeolites is possible microbiological contamination of zeolite sorbents used in the remediation of hazardous heavy metal-polluted soils (Shi *et al*., 2009) or in the purification of industrial wastewater (Martemianova *et al*., 2016). In such cases it is necessary to provide the sorption materials with bacteriostatic properties in order to prevent the growth of microorganisms on their surface.

To obtain ion exchangers saturated with transition metals, either synthetic zeolites of types A, X, and Y with high aluminum content (Kwakye-Awuah *et al*., 2008; Ferreira *et al*., 2012; Guerra *et al*., 2012; Demirci *et al*., 2014; Jiraroj *et al*., 2014; Milenkovic *et al*., 2017) or cheap natural clinoptilolite of different origin (Semmens & Seyfard, 1978; Semmens & Martin, 1988; Jama & Yücel, 1989; Zamzow & Schultze, 1995; Kawahara *et al*., 2000; Langella *et al*., 2000; Rivera-Garza *et al*., 2000; Top & Ülkü, 2004; De la Rosa Gomez *et al*., 2008; Hrenovic *et al*., 2012; Hrenovic *et al*., 2013; Akhigbe *et al*., 2014; Martemianova *et al*., 2016; Rossainz-Castro *et al*., 2016; Milenkovic *et al*., 2017) are applied. Ion exchange reactions are used to saturate the zeolite with transition metal ions. Usually ion exchange is performed by mixing grinded and sieved zeolite with the corresponding metal solution with a concentration up to 1 N in a weight ratio up to 1:100 and shaking or stirring the suspension at temperatures from room to 60 °C. Such a “wet” process takes from several hours to several days, in some cases a week or more, at the end of the process the suspensions are separated by filtration, the products are washed and dried. When using natural zeolites as a raw material, the process is usually carried out in two stages – first, a monocationic form is obtained (usually the sodium form), which is then converted into a bicationic form containing a transition metal. In general, the process consumes a large amount of both salt solution and distilled or deionized water for washing the product.

An alternative is a “dry” or “solid-phase” process of mixing a highly dispersed zeolite with the corresponding metal salt for several minutes, after which the resulting product is washed and dried. This method can be applied for both synthetic and natural zeolites as raw materials. In the latter case, it is possible to avoid pre-washing of raw materials, which also simplifies and reduces the cost of the process. Both methods of “ion exchange synthesis” involve the use of highly dispersed zeolite samples with a high specific surface to increase the area of contact with the source of counter ions.

The aim of our study was to develop a fast, environmentally friendly and economical method for producing zeolite with a fairly high content of silver and zinc considering utilization of the exchanged forms as an antibacterial material. Instead of the commonly used clinoptilolite having a relatively low ion exchange capacity (crystal chemical formula $|Ca_4(H_2O)_{24}|$**$[Al_8Si_{28}O_{72}]$-HEU**, scientific weight capacity (SWC) 3.08 meq/g), phillipsite ($|K_2\,(Ca,Na_2)_2\,(H_2O)_{12}|$ **$[Al_6Si_{10}O_{32}]$-PHI**, SWC = 5.09 meq/g) was used, the deposit of which is located in western Georgia.

2. Experimental

Preparation of the silver and zinc containing zeolite materials by “ion exchange synthesis” was carried out using Georgian natural phillipsite-containing tuff rock from Shukhuti (Western Georgia) and named as NPSH (natural phillipsite from Shukhuti). As stated earlier (Tsitsishvili *et al.*, 1998), the tuff of the Shukhuti deposit is characterized by a high content of the zeolite phase, about 60% for samples from the lower plot with main impurities of a zeolitic (clinoptilolite-heulandite) nature, and over 90% for samples from the upper plot containing clay minerals as main impurities and used in this study; presence of heulandite-clinoptilolite impurities was controlled by XRD peaks at $2\Theta = 9.84\text{-}9.87^o$ (hkl=020) and at $2\Theta = 22.2\text{-}22.7^o$ (hkl=131, 400, 300, 240).

Analytical grade silver nitrate $AgNO_3$ and zinc chloride $ZnCl_2$ were used as salts to introduce silver and zinc ions into the zeolite structure. Ion exchange was carried out as follows: crushed and sieved zeolite NPSH (particle size less than 63 μm) and the corresponding salt were mixed in weight ratios 1:1, 1:3 and 1:6, thoroughly mixed in an agate mortar for 5-10 minutes, depending on the cationic form. 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^{o}C in a thermostat; samples with a maximum silver content are labeled as Ag-PSH (silver-containing phillipsite), with a maximum zinc content called Zn-PSH (zinc-containing phillipsite).

Chemical composition of the natural phillipsite and ion-exchanged samples was determined by elemental analyses carried out using a Spectromom 381L plasma spectrometer and a Perkin-Elmer 300 atomic absorption spectrometer, as well as by energy dispersive X-ray (EDS) analysis. X-ray powder diffraction patterns were obtained from a DRON-4 diffractometer, employing the Cu-K_α line and scanning at 1^O per minute, FTIR spectra in the wavenumber range 4000-400 cm^{-1} were recorded on the Perkin-Elmer FTIR spectrometer (version 10.4.2) using the KBr pellet technique for sample preparation, SEM images were obtained by using Jeol JSM6510LV scanning electron microscope equipped with Oxford Instruments X-Max 20 analyzer for EDS.

Specific surface area and pore volume of the original and modified samples were determined by the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods measuring nitrogen adsorption-desorption on Micromeritics ASAP 2020 Plus physisorption analyzer. Water adsorption capacity was measured under static conditions (p/p_S=0.40, 20^{o}C).

The determination of the amount of silver and zinc released from modified zeolites Ag-PSH and Zn-PSH 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 hours after loading 0.1 gram of zeolite in 100 ml of salina.

The antibacterial activity of NPSH, Ag-PSH and Zn-PSH was tested against Gram-negative bacteria *Escherichia coli*. 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.

Luria Bertani (LB) medium sterilized by autoclaving (121^{o}C, 15 min) prior to the antibacterial activity tests was used as a growing medium, bacteria were grown aerobically in LB broth at 37°C for 12 hours, the culture was centrifuged twice (10,000 rpm), and the cells were washed and suspended in distilled water. 1 cm^3 of the prepared biomass suspension of approximately 10^7 colony-forming units per cm^3 (CFU/cm^3) was inoculated into the Schott's bottles with 100 cm^3 of autoclaved saline, and zeolite samples in a concentration of 0.1 g/100 cm^3 were added. The bottles were incubated in a thermostatic water bath for 24 h at 37 ± 0.1 °C with shaking at 105 rpm. The number of viable cells was determined taking 0.1 mL of water + bacteria + zeolite mixture at the beginning of the experiment and after 1 (short-term exposure corresponding to the lag phase of bacterial growth), 3, 6, and 24 (the stationary phase of bacterial growth) hours. The aliquots were diluted in distilled water, spread on LB agar plates and incubated at 37°C for 24 hours. Bacterial colonies were counted using microscope.

Bacteriostatic properties of natural and modified zeolite samples were determined by the disk diffusion (Kirby-Bauer) method in standard conditions (Bauer *et al*., 1966) using the culture of *E.coli* grown on Mueller–Hinton agar medium at 37^{o}C for overnight and placed (10^9 CFU/ cm^3) on Mueller–Hinton agar (3 mm deep) poured into 100 mm Petri dishes. 0.2 g of zeolite specimen in the form of pellets with 8 mm in diameter was placed into the plates. The plates were incubated at 37 ^{o}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.

All experiments on antibacterial activity of NPSH, Ag-PSH and Zn-PSH were done in triplicate. The values obtained were averaged to give the final data with standard deviations.

3. Results and discussion

The chemical composition was calculated on the basis of elemental analyses results for one quarter of the unit cell containing 32 oxygen atoms, as defined by the crystal chemical formula of phillipsite|$K_2(Ca,Na_2)_2(H_2O)_{12}$|[$Al_6Si_{10}O_{32}$]-**PHI** (Baerlocher *et al*., 2007). Chemical composition of natural phillipsite and its modified forms with a maximum silver or zinc content are listed in the Table 1 in terms of the empirical formulas $(M_aNa_{2b}K_{2c}Ca_dMg_e)Me_f[Al_xSi_yO_{32}]\cdot nH_2O$, where M = Ag^+ or ½Zn^{2+}, and impurity metals Me = $^1/_3Fe^{3+}$, ½Cu^{2+}, ½Mn^{2+}, etc.

Table 1. Chemical composition of natural, silver- and zinc-containing phillipsite

Sample	Empirical formula	Si/Al
NPSH	$(Na_{1.30}K_{2.0}Ca_{0.30}Mg_{0.25})Me_{0.10}[Al_{4.50}Si_{11.80}O_{32}]\cdot 11.4H_2O$	2.62
Ag-PSH	$(Ag_{3.40}Na_{0.01}K_{0.08}Ca_{0.25}Mg_{0.20})Me_{0.06}[Al_{4.45}Si_{11.20}O_{32}]\cdot 14.2H_2O$	2.52
Zn-PSH	$(Zn_{1.80}Na_{0.01}K_{0.34}Ca_{0.075}Mg_{0.125})Me_{0.05}[Al_{4.40}Si_{11.60}O_{32}]\cdot 15.6H_2O$	2.64

The Si/Al ratio and comparatively high calcium content (Ca/Na=2.1) of natural phillipsite indicates its hydrothermal origin; water content is in a good accordance with accepted crystal chemical formula.

The conventional mechanical grinding of tuff to obtain <63 μm (240 mesh) fraction leads to the formation of a multitude of micrometric crystallites (Fig. 1a), so that obtaining a highly dispersed fraction is not difficult. Apparently, this is due to the fact that large crystallites (with dimensions of about 50 μm) consist of smaller (about 5 μm) bound together by clay minerals (Fig. 1b).

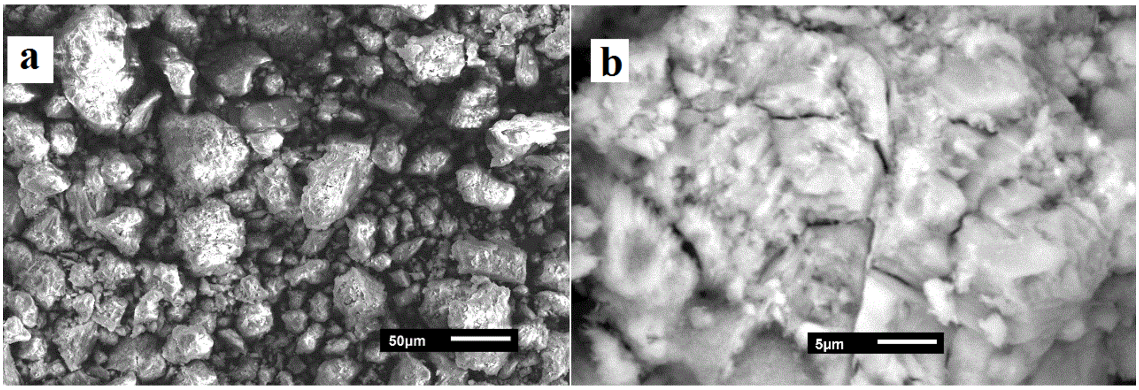

Figure 1. SEM images of NPSH

The high dispersion of the phillipsite sample NPSH used in solid-state ion-exchange synthesis is confirmed by the data on low-temperature sorption-desorption of nitrogen: the BET surface area is 73.5 m^2/g, total pore volume of pores less than 121 nm diameter is 0.2777 cm^3/g.

The calculated ion-exchange SWC of NPSH is 3.95 meq/g, which is lower than the theoretical value for the ideal structure of phillipsite, but higher than the values reported for natural clinoptilolites (Hrenovic *et al*., 2012; Akhigbe *et al*., 2014).

Ion exchange reactions followed by washing lead to the replacement of compensating alkaline and alkaline earth ions by silver or zinc ions and produce materials with different contents of silver and zinc, depending on the weight ratio of zeolite and corresponding salt, as well as on the duration of the process. Degree of replacement was calculated on the basis of elemental analyses results and is expressed as the ratio of Ag^+ or $½Zn^{2+}$ moles to the number of moles of aluminum (or total number of M^+, Na^+, K^+, $½Ca^{2+}$, $½Mg^{2+}$, and Me^+ ions). The conditions and results of the experiments are shown in Table 2; standard deviations are given in parentheses.

Table 2. Data on the ion-exchange synthesis of silver- and zinc-containing phillipsite

Reaction mixture	Weight ratio zeolite/salt	Duration of solid state reaction (min)	Degree of replacement (%)
NPSH–$AgNO_3$	1:1 1:3 1:6	5 10 10	81.7 (5.0) 84.0 (5.5) 85.5 (5.5)
NPSH – $ZnCl_2$	1:1 1:3 1:6	5 10 10	70.5 (3.5) 76.2 (4.0) 82.0 (4.8)

Silver ions Ag^+ quite easily enter the microporous structure of phillipsite, the introduction of zinc ions Zn^{2+} requires an increased amount of salt and a longer contact with the surface of the zeolite. This can probably be explained by a slight difference in the hydration character of the ions entering the pores of the zeolite. So, an "isolated" silver ion Ag^+ (radius 0.115 nm) is larger than Zn^{2+} ion (radius 0.074 nm), but the hydrated silver(I) ion contains four water molecules ($Ag(H_2O)_4^+$) in a linearly distorted tetrahedron configuration, while the hydrated zinc(II) ion contains six water molecules ($Zn(H_2O)_6^{2+}$) and has regular octahedral configuration (Persson, 2010).

The introduction of hydrated ions into the channels and pores of the zeolite is facilitated by the developed system of macro- and mesopores in the used phillipsite NPSH: adsorption-desorption average pore volume diameter by BET exceeds 15 nm, the BJH method gives adsorption average pore diameter of 22.0 nm, and desorption average pore diameter of 54.4 nm.

The system of mesopores remains after ion exchange, as evidenced by SEM images of modified samples (see Figures 2 and 3). In general, the procedure of ion-exchange synthesis leads to a significant increase in the dispersion of the material. As can be seen from the images, the size of the largest crystallites does not exceed 20 μm for Ag-PSH (Fig. 2a) and 30 μm for Zn-PSH (Fig. 3a). Compared with the starting material NPSH, the proportion of crystallites smaller than 2 μm is increased, especially for the Ag-PSH sample (Fig. 2b). The main difference between Ag-PSH and Zn-PSH samples is that the silver-containing crystals are sufficiently isolated, while the aggregation of zinc-containing crystallites is preserved to a greater degree, like in the natural phillipsite.

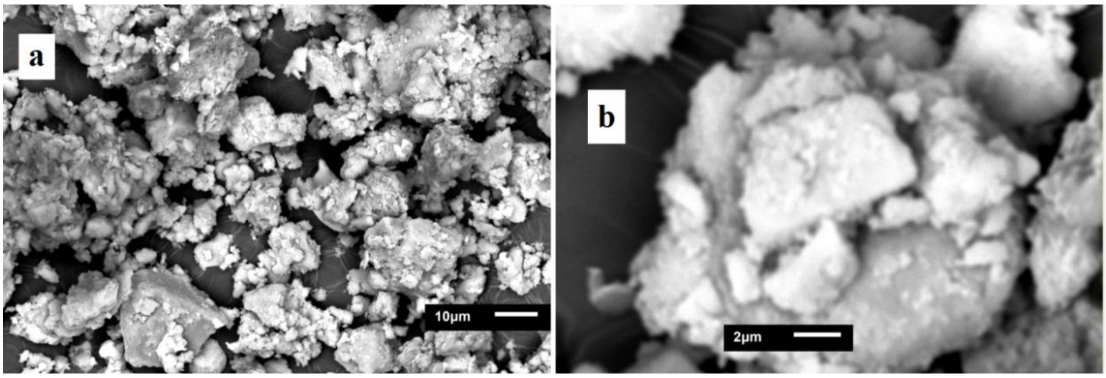

Figure 2. SEM images of Ag-PSH

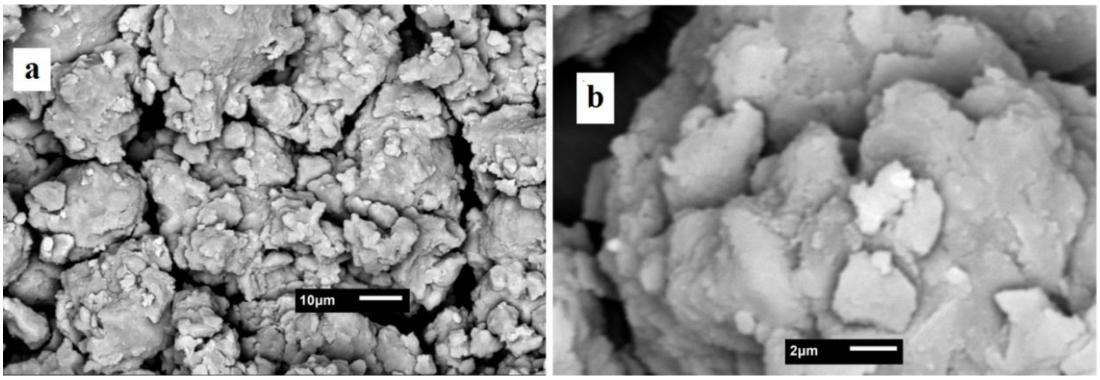

Figure 3. SEM images of Zn-PSH

According to the elemental analysis data (see Table 1), when silver and zinc ions are introduced into the phillipsite crystal lattice, potassium and sodium ions are mainly displaced. Degree of replacement (see Table 2) is quite high, the obtained modified forms contain a large amount of transition metals – up to 230 mg/g (2.14 mmol per 1 g of zeolite) of silver in Ag-PSH sample, and up to 86 mg/g (approximately 1.3 mmol per 1 g of zeolite) of zinc in Zn-PSH sample. In Table 3, our results are compared with the literature data on the content of silver and zinc in various natural clinoptilolites and synthetic zeolite.

Table 3. Metal content (mmol/g) after ion exchange in different zeolites

Exchanged zeolite	**Ag**	**Zn**
Natural phillipsite from Shukhuti	2.14	1.30
Clinoptilolite from Gördes, Turkey (Top & Ülkü, 2004)	1.85	1.04
Natural clinoptilolite (Akhigbe *et al*., 2014)	0.82	
Clinoptilolite from deposit Zlatokop, Serbia (Hrenovic *et al*., 2012)		0.225
Zeolitic tuff from Semnan deposit, Iran (Milenkovic *et al*., 2017)	0.24	0.24
Synthetic Linde 4A (Milenkovic *et al*., 2017)	0.27	0.28
Synthetic faujasite (Jędrzejczyk *et al*., 2017)	0.14	

It is obvious that phillipsite is a more promising carrier of silver and zinc than natural clinoptilolite. The low content of transition metals in synthetic zeolites is not surprising. The Linde 4A (crystal chemical formula $|Na_{12}(H_2O)_{27}|_8[Al_{12}Si_{12}O_{48}]_8$-**LTA**) was designed for water softening, this zeolite is characterized by high aluminum content and is considered as an ideal ion exchanger, but this is true only for $2Na^+ \leftrightarrow Ca^{2+}(Mg^{2+})$ reactions, the exchange of monovalent ions with cumulating of Ag^+ in crystal lattice is unlikely, as is the exchange of sodium with a strongly hydrated zinc ion. In the structure of faujasite (crystal chemical formula $|(Ca,MgNa_2)_{29}(H_2O)_{240}|[Al_{58}Si_{134}O_{384}]$-**FAU**), there are relatively few aluminum atoms and quite a lot of calcium and magnesium, which hardly enter into an exchange with ions in solution.

Unfortunately, there is no data on the chemical composition of silver-loaded zeolite X (Kwakye-Awuah *et al*., 2008), the presence of silver is confirmed by EDX spectrum, but its content is not indicated.

X-Ray powder diffraction patterns of natural and modified phillipsites have been assigned on the basis of pattern from the IZA database of zeolite structures[1] calculated for $|K_2CaNa_2(H_2O)_{12}|[Al_6Si_{10}O_{32}]$-**PHI** structure.

The calculated pattern includes 190 peaks, and it was simplified as follows: peaks with a relative intensity of less than 10% were not taken into account, and if two or more peaks differed in the 2Θ angle by less than 0.15 degree, then only one peak with the greatest intensity was taken into consideration. For example, in the X-ray powder diffraction pattern of natural phillipsite the strongest peak at $2\Theta = 27.864^o$ (hkl = 041) is followed by two overlapping peaks at $2\Theta = 27.865^o$ (131 and –302) with lesser intensity (7.77 and 18.24%) excluded from consideration. A simplified pattern is listed in Table 4 (hkl indexes, 2Θ angles, d-spacings, and relative intensities) along with data on relative intensities for modified zeolites.

The simplified pattern is in good agreement with the simulated pattern of Ca-phillipsite from the RRUFF base (Lafuente *et al*., 2015) and with the pattern of NPSH.

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[1] Database of Zeolite Structures of the International Zeolite Association:http://www.iza-structure.org

Table 4. Calculated XRD pattern and experimental XRD data for original and modified phillipsites

Simplified calculated XRD pattern				**Experimental relative intensities (%)**		
Hkl	**2Θ (^{o})**	**d (Å)**	**Relative intensity (%)**	**NPSH**	**Ag-PSH**	**Zn-PSH**
–101	10.920	8.0952	18.47	<10	n.d.*	<10
001	12.336	7.1691	70.87	56	<15	73
011	13.806	6.4088	31.74	23	35	18
120	16.471	5.3774	31.70	<10	<10	45
021	17.503	5.0626	41.30	36	24	28
–201	17.969	4.9323	69.46			35
–211	19.018	4.6628	11.11	n.d.	n.d.	<10
111	21.491	4.1314	42.33	15	36	24
–112	21.623	4.1065	44.60		59	61
–221	21.873	4.0600	25.02	n.d.	n.d	<10
140	27.211	3.2745	38.42	35	33	40
022	27.818	3.2044	58.14	60	55	52
041	27.864	3.1993	100.00	100	100	100
–311	28.466	3.1329	61.52	53	42	37
–321	30.493	2.9291	21.69	<10	32	28
102	32.413	2.7599	21.50	46	40	<15
102	33.028	2.7099	26.46	48	36	23
–223	33.433	2.6779	23.11	16	<10	16
042	35.433	2.5313	15.05	n.d.	<10	n.d.
–341	37.622	2.3888	13.96	15	n.d.	
–252	38.511	2.3358	11.16	n.d.		

* not detected

The IR spectrum of natural phillipsite NPSH is similar to the spectra of sodium and calcium phillipsites from the RRUFF database[2]. Assignment of the main bands in mid infrared was made on the basis of general principles of infrared structural studies of zeolite frameworks (Flanigen *et al*., 1971), results are given in the Table 5.

Table 5. Assignment of peaks (cm^{-1}) in infrared spectra of natural and modified phillipsites

Vibration modes	**NPSH**	**Ag-PSH**	**Zn-PSH**
Asymmetric stretching of OH group	3436 br*	3530 br	3570 br
Bending vibration of H–OH	1647	1652	1651
Bending vibration of bridging –OH-O–	1520 br	1495 br	1463 br
Internal asymmetric stretching	1200 sh**	1100 sh	1100 sh
External asymmetric stretching (ν_{asym})	1030	1031	1034
External symmetric stretching	760 sh	825	790 sh
Internal symmetric stretching	694	698	696
Double ring vibration	606; 536	608; 536	594; 532
Internal T-O bending vibration (δ)	444	440	442
Absorbance (ν_{asym})/absorbance (δ)	1.82	1.80	1.75

* broad peak; ** shoulder

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[2] RRUFF^{TM} Project (http://rruff.geo.arizona.edu): Na-phillipsite http://rruff.info/phillipsite/R070271;Ca-phillipsite http://rruff.info/phillipsite/R050078

No notable changes were observed in the IR spectra of the modified phillipsites as compared with the vibration bands of raw zeolitic mineral. In the spectra of the modified samples, the intensity of the broad band at 3200 – 3700 cm^{-1} is increased due to the large number of water molecules in the samples containing silver and zinc.

For any zeolite, two groups of vibrations are considered due to topology and the mode of arrangement of the structure: internal bending vibrations of T–O (T=Si, Al), and vibrations of external linkages between the SiO_4 and AlO_4 tetrahedrons (Flanigen *et al*., 1971). The ratio of the absorbance of asymmetric stretching vibration of the external tetrahedron with frequency ν_{asym} to the absorbance of internal bending vibration with frequency δ was used for the evaluation of the IR spectra data for natural and modified Mexican zeolite (Rivera-Garza *et al*., 2000).

The authors showed that for a clinoptilolite-heulandite mixture and its metal-containing forms, this ratio varies from 1.34 to 1.64, but for phillipsite NPSH and its modifications, this ratio varies only slightly.

The absence of the vibration band of NO_3^- (1350 – 1400 cm^{-1}) in the spectra of clinoptilolite treated with a silver nitrate solution made it possible to conclude that Ag is into the zeolite network occupied ion exchange sites (Rivera-Garza *et al*., 2000). The same conclusion can be made for the silver-containing phillipsite Ag-PSH. In the same time, in insufficiently well-washed silver-enriched phillipsite samples, a narrow absorption band is observed at 1385 cm^{-1}, which can be attributed to NO stretching vibrations characteristic for nitro compounds. This effect can be used to monitor the purity of silver-containing samples.

Both XRD and FTIR data show developed zeolitic crystal microporous structure in metal-containing samples, and it has been confirmed also by comparatively high averaged value of water adsorption capacity under static conditions at the “plateau” pressure (see Table 6).

Table 6. Comparison of water sorption (p/p_s=0.4, 20°C) by different natural and modified phillipsites

Sample	**Water sorption**	
	mmol/g	cm^3/g
Phillipsite from lower plot of Shukhuti (Tsitsishvili *et al*., 1998)	5.21	0.094
Natural phillipsite from Nevada, USA (Tsitsishvili *et al*., 1998)	6.50	0.117
NPSH	7.25	0.131
Ag-PSH	5.54	0.100
Zn-PSH	6.55	0.118

The relatively low adsorption capacity of two natural phillipsites is associated with a low content of the zeolite phase, about 60% for the sample from the lower plot of Shukhuti deposit, and about 80% for the natural phillipsite from Nevada.

The decrease in the adsorption capacity in metal-containing samples is explained by the fact that hydrated shells of $Ag(H_2O)_4^+$ and $Zn(H_2O)_6^{2+}$ ions occupy a certain adsorption volume in the cavities and pores of phillipsite.

The release (leaching) of these ions into the salina solution occurs over time, the measurement results for zeolites containing 230 mg/g of silver or 86 mg/g of zinc are presented in Table 7.

The amount of silver ions released after 6 hours corresponds to their concentration of 0.067 mM, which is higher than the minimal inhibitory concentration

(MIC) value for silver ions toward *E. coli*, 3.996 mg Ag in dm^3 (Mulley *et al*., 2014) or 0.037 mM.

Table 7. Leaching (mg/L) of silver and zinc ions from modified phillipsites

Ion	**The exposure time (hours)**				
	0	1	3	6	24
Ag^+	0	<5	<5	7.2±1.5	20.6±4.5
Zn^{2+}	0	5.2±1.2	12.7±2.5	23.0±5.5	32.4±6.6

On the contrary, the amount of zinc ions released after 24 hours corresponds to their concentration of 0.48 M, which is lower than MIC value for zinc ions toward *E. coli*, 1 mM (Navarro *et al*., 2013).

Despite this, both modified samples exhibit a certain antibacterial activity. Table 8 shows the relative number of viable cells of *E. coli* suspended in water after their contact with natural and modified phillipsites in relation to the number of cells at the beginning of the experiment.

Table 8. The change in the relative number of viable cells of *E. coli* contacting with natural and modified phillipsites in time

Phillipsite	**Relative number of viable cells of *E. coli* (%)**				
	At the beginnig	**after 1 h**	**after 3 h**	**after 6 h**	**after 24 h**
NPSH	100	102.5±4.2	98.3±3.5	99.6±4.5	100.5±4.8
Ag-PSH	100	72.0±4.1	23.6±2.8	0	0
Zn-PSH	100	93.2±6.5	51.6±4.7	30.4±2.2	0

Taking into account the leaching of bioactive metals and their comparison with the values of the minimal inhibitory concentrations, we can conclude that the antibacterial activity of Zn-PSH could be ascribed to the metal-containing zeolite M-Z itself and not to the leached metal ions. The silver-containing zeolite Ag-PSH also exhibits a certain antibacterial activity even before the concentration of ions in the solution reaches the MIC value, and its bactericidal effect could be ascribed not only to released Ag^+ ions but also to Ag-PSH itself.

The total number of bacteria in bottles with NPSH was not significantly different than in the corresponding controls, showing that natural and modified phillipsite had no antibacterial activity itself.

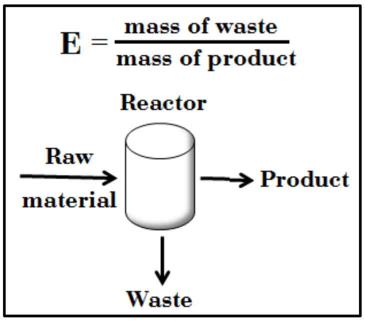
Results of the Kirby-Bauer test are given in the Table 9. No antibacterial action was observed for the original phillipsite.

Table 9. Bacteriostatic properties of natural and modified phillipsites

Sample	**Inhibition zone diameter (mm)**
Petri dish with *E. coli* only (check)	0 – confluent growth
Petri dish with *E. coli* and NPSH	0 – confluent growth
Petri dish with *E. coli* and Ag-PSH	18.6±0.7
Petri dish with *E. coli* and Zn-PSH	16.3±0.9

The width of the inhibition zones of the antibacterial metal-exchanged forms Ag-PSH and Zn-PSH is of the same order of magnitude as for silver- and zinc-containing clinoptilolite forms (Top & Ülkü, 2004).

Modern production of drugs must comply with environmental standards and principles. The use of synthetic and natural zeolites for the production of carriers of medicinal metals meets the conditions of wasteless production in relation to the main raw materials and reagents, but the consumption of associated materials is rather high.



An important environmental and green chemistry metrics is Sheldon's factor E, the ratio of the mass of waste per mass of product (Sheldon, 2007), and reduction of this factor is an urgent task.

Table 10 presents data on the consumption of liquid materials for various methods of ion-exchange production of silver or zinc containing zeolites. Information about the required quantities of zeolites, reagents and solutions have been obtained from published works (Hrenovic *et al.*, 2012; Akhigbe *et al.*, 2014; Jędrzejczyk *et al.*, 2017; Milenkovic *et al.*, 2017; etc.). Data on the amount spent on washing the target products by distilled water, as a rule, are not given in publications, but this expense was estimated on the basis of our experience (Tsitsishvili *et al.*, 2017). So, for washing one gram of product filtered from a solution, you need at least 300 mL of water, and washing after a "dry" ion exchange requires at least 500 mL. Solid waste zeolites, reagents, packaging and other consumables were not taken into account.

Table 10.Waste generated in the production of one gram of metal-containing zeolite andSheldon's factor E

Ion exchange method	**Liquid waste (mL)**		**E**
	Salt solution	Water	
Solid state ion exchange on phillipsite	–	500	500
Ion exchange on synthetic zeolite in solution	>400	300	>700
One-step ion exchange on clinoptilolite in solution	>500	300	>800
Two-step ion exchange on clinoptilolite in solution	>1000	600	>1600

The findings suggest that the "dry" method is preferable to the "wet" method.

4. Conclusion

As a result of the conducted research, it was established that by solid-state ion-exchange reactions between natural phillipsite and corresponding salt, zeolite materials can be obtained with a significantly higher content of silver (up to 230 mg/g) and zinc (up to 86 mg/g), rather than obtained by ion exchange in solutions on synthetic zeolites and natural clinoptilolite.

The materials obtained retain the microporous crystal structure of phillipsite and its adsorption properties; they are characterized by a developed system of mesopores and have a high degree of dispersion, and also exhibit bactericidal and bacteriostatic activity towards Gram-positive bacteria *Escherichia coli*.

Preparation of metal-containing zeolite materials by dry synthesis produces less wastewater than similar production by ion exchange in solutions.

Consequently, considering physical-chemical properties of obtained materials, compliance of proposed method with high environmental standards, and antibacterial activity results, Georgian phillipsite from Shukhuti, upper plot, can be proposed as a low cost raw material for manufacturing of antibacterial products.

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