The aim of this work was the preparation, characterization and antibacterial activities testing of copper and silver doped Romanian zeolite. The structural and morphological characterization of copper and silver doped zeolite versus natural zeolite was performed using Laser Induced Breakdown Spectroscopy (LIBS), Scanning Electron Microscopy (SEM) and Infrared Spectroscopy (IR). In addition, natural zeolite doped with copper and silver was electrochemically characterized by using cyclic voltammetry (CV) with boron-doped diamond electrode (BDD). The antibacterial activities testing of the two oligoelement doped zeolite was achieved on a real stagnant water sample that belongs to residential area of Timisoara, Romania, studying some microbiological parameters as total coliforms, thermotolerant coliforms (faecal coliforms) and enterococcus (streptococcus faecalis).

Keywords: silver doped zeolite, copper doped zeolite, antibacterial activity, electrochemical characterization

Modern water treatment technologies have been responsible for negative impact on human health, because of some toxic and carcinogenic disinfection by-products. Using some materials based on oligoelements modified zeolite could be regarded as an alternative process of drinking water disinfection.

Natural zeolites attract a lot of attention in many applications due to their low cost, abundant deposits and selectivity for a large number of cations [1,2]. In this context, natural clinoptilolite, which is formed in abundance, is one of the most appreciated due to its non-poisonous nature and great accessibility [3].

The choice of copper and silver ions for doping the zeolitic material are based on well-known antibacterial effects of these oligoelements, but their using as CuSO₄ and AgNO₃ form can unfavorably affect the human health quality. The antimicrobial activity of modified zeolite with inorganic materials (Ag, Cu) depends on the mineral carrier, e.g. zeolite type, as interaction between zeolite and inorganic material [4,5]. It has been found that for the uniform retaining of the copper and silver ions with antibacterial property, the zeolite must exhibit a SiO₂/Al₂O₃ molar ratio at most 14, this molar ratio being ranged between 8.5 to 10.5 for clinoptilolite [6]. Taking into account these aspects, in this paper it was used natural zeolite from Mirsid, Romania, with 68 % wt. clinoptilolite, for obtaining the zeolite doped with silver and copper.

This work aimed to the preparation, characterization and antibacterial effect of zeolite doped with copper and silver. The obtaining methods of zeolites modified with copper and silver were selected based on the consideration that the element amount retained into/on zeolite lattice has to be smaller than the total retaining capacity in order to enhance the antibacterial activity. The structural and morphological characterization of the zeolite doped with copper and silver compared with natural zeolite was achieved with Laser Induced Breakdown Spectroscopy (LIBS), Scanning Electron Microscopy (SEM) and Infrared Spectroscopy (IR) techniques.

The antibacterial activity of the zeolite doped with silver or copper requires avoiding the presence of reduced forms of silver and copper into zeolite. The electrochemical studies, e.g. cyclic voltammetry, should be used to assess the redox behaviour of the species forms, which are encapsulated within zeolite. The Romanian natural zeolite doped with silver and copper was characterized electrochemically using CV and BDD electrode.

The antibacterial activities testing for the two oligoelment doped zeolite was realized on a real stagnant water sample that belongs to residential area of Timisoara, Romania, studying some microbiological parameters: total coliforms, thermotolerant coliforms (faecal coliforms) and enterococcus (streptococcus faecalis).

Materials and methods
Obtaining of doped material
The Romanian zeolitic mineral from Mirsid, was supplied by Cemacon Company, Romania. The mineral was powdered and sieved with a Multilab sieve shaker. The diameter of grains size selected to carry out the experiments was between 315-500 µm.

The preparation of Ag and Cu doped zeolite was realized by mixing natural zeolite with 0.1 M AgNO₃ solution (2 hours) and 0.05 M CuSO₄ solution (5 h) [6]. The Ag and Cu doped zeolite obtaining (Z-Ag, Z-Cu) was realized by the thermal treatment of Ag and Cu modified zeolite at 500°C.

Physico - chemical and morphological characterization of zeolite
To determine the total retaining capacity of copper and silver doped zeolites the following method was used: 1.000 g of zeolite with the dimension of grains between 315-500 µm was shaken with 25 mL of 0.05 M CuSO₄ and respectively, 0.1M AgNO₃ during 14 days [7].

To determine the presence of copper and silver within zeolite lattice, the samples were analyzed by Laser Induced Breakdown Spectroscopy (LIBS). The experimental arrangement and conditions for LIBS use was presented.

* email: corina.orha@chim.upt.ro

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The obtaining methods of zeolites modified with copper and silver were selected based on the consideration that the element amount retained into/on zeolite lattice has to be smaller than the total retaining capacity in order to enhance the antibacterial activity. The structural and morphological characterization of the zeolite doped with copper and silver compared with natural zeolite was achieved with Laser Induced Breakdown Spectroscopy (LIBS), Scanning Electron Microscopy (SEM) and Infrared Spectroscopy (IR) techniques.
by L. Paksy et al [8]. The material ablation and the excitation were performed with Q-switched Nd:YAG laser with an energy of 12-15 mJ, using an Cu and Ag standards. The Ag and Cu amount into zeolite lattice was determined by using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) after sample mineralization. The ICP-AES analysis was made with an ICP-AES SpectroFlame spectrometer.

The morphology and the composition of the unmodified/modified zeolite were characterized by using Scanning Electron Microscopy (SEM) and Infrared Spectroscopy (IR). The SEM images were made in a Microscop Electronic Inspect S FEI Company, The Netherlands, for copper doped zeolite, and for natural and silver doped zeolite in a Philips XL-20 microscope. The IR spectra were recorded in KBr pellet for solid compounds with a Jasco FT/IR-430 instrument.

The electrochemical characterization of Ag and Cu doped Romanian zeolite

The electrochemical behaviour was performed by three repetitive cyclings between -0.5 V to 1V vs. SCE in a 0.1 M Na₂SO₄ supporting electrolyte, Romanian natural zeolite suspended in 0.1 M Na₂SO₄ solution, Romanian Ag and Cu doped zeolite suspended in 0.1M Na₂SO₄ solution. The ratio between zeolite and 0.1 M Na₂SO₄ solution was 1g zeolite: 100 ml 0.1 M Na₂SO₄ solution. All electrochemical measurements were carried out using a potentiostat/galvanostat PGSTAT 302 (Eco Chemie, The Netherlands) controlled with GPES 4.9 software and a three-electrode cell, with a saturated calomel electrode (SCE) as reference electrode, a platinum counter electrode, and boron-doped diamond (BDD) working electrode. Sodium sulphate was analytical grade from Merck, and freshly prepared with double-distilled water. BDD electrode was supplied by Windsor Scientific Ltd., United Kingdom.

Results and discussion

Physico-chemical and morphological characterization of the zeolite

The total retaining capacity of natural zeolite comparative results for silver and copper are presented in figure 1.

The total retaining capacity of natural zeolite for silver was about 0.1 mg Ag/g zeolite and for copper 0.02 mg Cu/g zeolite. The copper retaining occurred with slower rate compared with silver, and the retaining capacity of the natural zeolite for copper was about five times lower than for silver. The better selectivity of zeolite for silver could be due to both the result of the high polarizability of silver cation because of the charge density and to the probability of the adsorption process of silver oxide on the zeolite surface.

The qualitative assessment of the two elements in zeolite lattice is shown by the laser spectrum (fig. 2). The copper and silver amounts retained within zeolite lattice after thermally treatment and determined by ICP-AES are presented in table 1.

Antibacterial activities tested

One gram of silver and copper doped zeolite was put in contact with 1000 ml real stagnant water sample. Samples were kept in refrigerator (-4°C) and the maximum contact time was 45 days. For the detection of Coliform organism were used Lauryl Sulfate Broth and Azide Dextrose Broth (Rothe) for detection of Fecal Streptococci. The culture medium was provided by Mediclin, Romania.

The total coliform presence is determined based on Romanian standard by using the presumption test by sample or/and serial dilutions inoculation. The test is carried out into a number of reached medium containing tubes. The positive reaction is diagnosed by confirmation test on solid medium, at the temperature of 37°C±0,5°C, for 48 h. The multiple tube techniques yield the statistically derived Most Probable Numbers of organisms per aliquot (usually 100 ml) of water [9].
The crystals of natural zeolite presents monoclinic symmetry characteristic to blades with well-defined edges, which is according to the literature [10,11].

SEM image of silver doped zeolite (fig. 4a) illustrates some agglomerated small crystals, with less well-defined edges than natural zeolite. In the case of copper doped zeolite, SEM image confirm the hypothesis of the copper oxide formation, emphasized by amorphous aspect of the surface (fig. 4b). The copper oxide appearance in the zeolite lattice after thermal treatment is necessary because these copper species are more reactive and improve the catalytic activities of the copper modified zeolite [12].

Figure 5 shows the comparative infrared spectra for silver doped zeolite (Z-Ag), copper doped zeolite (Z-Cu) and natural zeolite (Z-N).

The silver and copper doped zeolite IR spectra show some differences characteristic to the zeolite lattice differences in the vibration bands between 400-800 cm\(^{-1}\). At about 2300 cm\(^{-1}\) wavelength a peak corresponding to silver doped zeolite can be noticed. From IR spectra, it can be seen that silver doped zeolite lost the molecule of water-coordinated bonded to the zeolitic channels edges in great proportion versus copper doped zeolite.

The crystallinity of the samples was estimated by using the IR optical density ratio. According to the literature [13], it was calculated the optical density ratios of the 464 cm\(^{-1}\) (internal tetrahedra bending) and 606 cm\(^{-1}\) (external tetrahedra double ring) bands of Z-N, Z-Cu and Z-Ag as 0.76; 0.77; 0.77. The almost similar ratios suggest that the zeolite framework of all the samples remains intact.

Electrochemical characterization of silver and copper doped Romanian zeolite

In figure 6 is shown the comparative cyclic voltammograms of BDD electrode obtained in 0.1 Na\(_2\)SO\(_4\) supporting electrolyte, Romanian natural and Ag doped zeolite suspensions.

It has underlined that electrochemical response of BDD electrode in the presence of Romanian natural zeolite is the same as in the presence of 0.1 M Na\(_2\)SO\(_4\) supporting electrolyte, without new Faradaic signals. In the presence of Romanian Ag doped zeolite the cyclic voltammogram of BDD electrode presents two cathodic peaks at -0.36 V and 0.22 V, and one anodic peak at about 0.4 V versus SCE, the last two corresponding to the reduction and oxidation processes of Ag(I)/Ag couple. In accordance with the literature [14], the cathodic peak recorded at more negative potential (-0.36V/SCE) could be attributed to the reduction process of Ag clusters with charge excess and with different redox abilities.

The first scan results of CV gave information about the Cu species types at BDD electrode/Cu doped zeolite/ 0.1 M Na\(_2\)SO\(_4\) interface (fig. 7). The lack of the anodic peak during the anodic scanning and the presence of the cathodic peak, which occurred at potential value about -0.2 V vs. SCE, and correspond to the reduction of Cu (II) to Cu (I) proved the Cu (II) presence into BDD electrode/Cu doped zeolite/ 0.1 M Na\(_2\)SO\(_4\) interface. This is a desired aspect in order to apply Cu doped zeolite as antibacterial agent.

Antimicrobial activities testing

The microbiological characterization of a real stagnant water sample that belongs to residential area of Timisoara city, Romania, demonstrates the presence of a great number of total coliforms, thermotolerant coliforms (faecal...
coliforms) and a reduced number of enterococcus (streptococcus faecalis), and are presented in table 2.

Table 2
INITIAL MICROBIOLOGICAL CHARACTERIZATION OF WATER SAMPLE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of viable cells of total coliforms/100 ml</th>
<th>Number of viable cells of thermotolerant coliforms (faecal coliforms)/100 ml</th>
<th>Number of viable cells of enterococcus (streptococcus faecalis)/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>&gt; 1690</td>
<td>&gt; 1690</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3 shows the removal efficiency of microbiological parameters of the silver and copper zeolitic mineral.

Table 3
THE BACTERIA REMOVAL EFFICIENCY OF MICROORGANISMS OF SILVER AN COPPER DOPED ZEOLITE

<table>
<thead>
<tr>
<th>Contact time [days]</th>
<th>Zeolite</th>
<th>Removal efficiency for total coliforms (%)</th>
<th>Removal efficiency for thermotolerant coliforms (faecal coliforms) (%)</th>
<th>Removal efficiency for enterococcus (streptococcus faecalis) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Z-Ag</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Z-Ag</td>
<td>85.79</td>
<td>99.70</td>
<td>100</td>
</tr>
<tr>
<td>31</td>
<td>Z-Ag</td>
<td>99.88</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>Z-Ag</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>Z-Cu</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Z-Cu</td>
<td>0</td>
<td>94.43</td>
<td>100</td>
</tr>
<tr>
<td>31</td>
<td>Z-Cu</td>
<td>85.79</td>
<td>99.64</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>Z-Cu</td>
<td>99.17</td>
<td>99.70</td>
<td>100</td>
</tr>
</tbody>
</table>
From table 3, it can be seen that for the silver doped zeolite application, the removal efficiency is 100% after 31 days of thermotolerant coliforms faecal coliforms contact time, and for total coliforms, the efficiency reached 100% after 45 days as contact time. For copper doped zeolite the removal efficiency of total coliforms was about 99.17% and for the thermotolerant coliforms (faecal coliforms) 99.70%.

The enterococcus (streptococcus faecalis) removal efficiency for the both materials was 100% after 7 days of contact time, but it must be taken into account the small amount of these microorganisms in raw water (table 2).

The antibacterial activity of doped zeolite was subjected to some parameters, i.e., ion type (Ag or Cu) and contact time.

Conclusions
Copper and silver doped zeolite was obtained and characterized for its use to obtain some materials with antibacterial activity.

The total retaining capacity of natural zeolite for silver was about 0.1 mg Ag/g zeolite and for copper was 0.02 mg Cu/g zeolite. The qualitative presence of silver and copper in zeolite lattice was determined by LIBS, and the quantitative presence was determined by ICP-AES.

The copper and silver doped zeolite morphology was influenced by the presence of both cations. The copper doped zeolite shown an amorphous shape, which suggest the presence of copper oxide with a high catalytic activity.

The electrochemical characterization of these doped zeolites using cyclic voltammetry technique on the BDD electrode proved the ions forms of Ag and Cu species encapsulated into zeolite, which are responsible for the antibacterial activity.

The Romanian silver and copper doped zeolite exhibited the antibacterial activity for the total removal total of coliforms, thermotolerant coliforms (faecal coliforms) and enterococcus (streptococcus faecalis) from a real stagnant water of a residential area of Timisoara city, Romania. The antibacterial activity was more effective for Ag doped zeolite, the removal efficiency of each indicator reached 100% after 45 days of contact time.

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