

Sorption Preconcentration of Silver for Atomic Absorption Analysis and Antibacterial Properties of the Acid-modified Clinoptilolite – Ag composite

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Sorptive properties of the acid-modified Transcarpathian clinoptilolite towards Ag(I) were studied under dynamic conditions. It was found that the most effective acid-modifier is 1 M HNO₃ solution. The sorption capacity value of H-clinoptilolite under the optimal conditions is 6.15 mg of Ag per 1 g of zeolite. The solid-phase extraction procedure with acid-modified clinoptilolite was used to pre-concentrate trace amounts of silver ions in aqueous solutions and then finally to determine them by the atomic absorption method. Due to the acceptable recoveries (≥ 98%) and RSD values (2.3–4.7%) for tap and lake water, the developed method can be successfully applied for the determination of trace amounts of silver ions in the presence of major components of water. It was established that the initial form of clinoptilolite has a better antibacterial effect against E. coli than the H-form. However, antagonistic activity against S. aureus was lower than against E. coli in both forms. The combination of different forms of clinoptilolite with Ag(I) increases their antibacterial activity.

Keywords: acid-modified clinoptilolite, preconcentration, silver, solid-phase extraction, antibacterial activity

Сорбційне концентрування срібла для атомно-абсорбційного аналізу та антибактеріальні властивості композиції кислотномодифікований клиноптилоліт – Ag

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Досліджено сорбційні властивості кислотномодифікованого закарпатського клиноптилоліту стосовно Ag(I) у динамічних умовах. Встановлено, що найбільш ефективною кислотою для модифікації є 1 М розчин HNO₃. Значення сорбційної ємності H-клиноптилоліту в оптимальних умовах становить 6.15 мг на 1 г цеоліту. Методика твердофазної екстракції з кислотномодифікованим клиноптилолітом використана для концентрування слідових кількостей йонів срібла у водних розчинах з наступним визначенням їх атомно-абсорбційним методом. Внаслідок прийнятних виходів (≥98%) та значень відносного стандартного відхилення (2.3–4.7%) для водопровідної та озерної води, розроблений метод може бути успішно використаний для визначення слідових кількостей йонів срібла у присутності основних компонентів води. Встановлено, що вихідна форма клиноптилоліту виявляє кращий антибактеріальний ефект стосовно E. coli, ніж H-форма. Щоправда протидіюча активність стосовно S. aureus була нижчою, ніж стосовно E. coli, для двох форм цеоліту. Комбінація різних форм клиноптилоліту із Ag(I) збільшує їх антибактеріальну активність.

Ключові слова: кислотномодифікований клиноптилоліт, концентрування, срібло, твердофазна екстракція, антибактеріальна активність

Silver belongs to the physiologically active components of mineral waters. Usually the concentration of this metal in natural waters containing silver is not high. At the same time even the scanty concentrations of silver exhibit the antibacterial activity. During the analysis of waters the previous preconcentration, exclusion and/or separation of silver trace amounts often need to be carried out [1-5]. For this purpose the solid-phase extraction (SPE) method is used quite often. The effective sorbents of heavy metals are the modified high dispersed silica [5-10], polyurethane [5], clays [11], zeolites [3, 6, 11-23]. The intensive investigations of natural zeolites sorptive properties towards Ag(I) have been carried out recently [14-18]. Unlike most of aluminosilicates these natural nanomaterials exhibit high sorption ability at a low concentration of a substance being sorbed. The uniqueness of natural zeolites is caused not only by the high efficiency and selectivity of sorption processes towards heavy metals, but also by wide possibilities of carrying out the modification of these minerals in order to change their properties in the desired direction. It is known [19, 23-29], that the H-form of clinoptilolite as the most common natural zeolite differs significantly from the unmodified clinoptilolite in sorption properties and in most cases it exhibits higher sorption capacity towards heavy metal ions. Acid-modified clinoptilolite is also an efficient sorbent of pharmaceutically active compounds (dexamethasone, venlafaxine, bupropion) from aqueous solutions and biosubstrates [30-32]. The best method to obtain the H-form of clinoptilolite, i.e. to activate it as a sorbent, is processing of this natural aluminosilicate with acid solutions.

Among inorganic antimicrobial agents silver takes a particular place. Silver ions act against bacteria cells in several directions at the same time which practically prevents the formation of a resistivity to silver remedies. The development of antimicrobial remedies with the application of sorbed silver causes a particular interest. This is due to the fact that silver supplies a powerful bactericidal effect, but at the same time it does not move into the water and biological liquids and, respectively, the excess of silver ions is not formed in water or blood. The zeolite composites with silver cause an increasing interest from the point of view of their antimicrobial action [3, 14-16, 18, 28, 29]. Zeolite samples, in particular, the "clinoptilolite-Ag" composite, which exhibit absorptive, catalytic and antimicrobial properties, are applied to utilize pesticides, chemical weapon and bacteriological remedies [28, 29]. In our previous research [3] a strong antimicrobial effect of a natural form of Transcarpathian clinoptilolite composite with Ag(I) towards gram-negative bacteria and yeasts was found.

Our goal is to investigate the sorptive properties of the acid-modified Transcarpathian clinoptilolite towards trace quantities of Ag⁺ in aqueous solutions in order to develop the method of Ag(I) preconcentration in a solid phase extraction mode with the further

determination using the atomic absorption method, and to study the antibacterial action of Ag–H–clinoptilolite composite samples.

Materials and Methods

Reagents. The clinoptilolite used in this investigation was obtained from the deposit near the village of Sokyrmysia in the Zakarpattia region of Ukraine. The preliminary analysis has shown that the main component was present at 85–90%, the specific surface area, determined by water sorption [33], was 59 m²g⁻¹. The chemical composition of Transcarpathian clinoptilolite is (in wt %): SiO₂, 67.29; TiO₂, 0.26; Al₂O₃, 12.32; Fe₂O₃, 1.26; FeO, 0.25; MgO, 0.99; CaO, 3.01; Na₂O, 0.66; K₂O, 2.76; H₂O, 10.90 [34].

The zeolite samples were grained in a ball-mill, the grain fraction (0.20–0.31) mm was selected, washed with distilled water and dried at room temperature.

Acid-modified clinoptilolite was obtained in the following way. The grained sample of zeolite (6 g) was pre-treated with 200 mL of 1 M HNO₃ for 24 h at the temperature of 20±1 °C. After separation from acid solution the sorbent was washed properly in distilled water. The H-form of clinoptilolite was dried at room temperature.

The H-clinoptilolite samples were calcined at the appropriate temperatures for 2.5 h in a drying oven WSU200 (Germany) and muffle furnace SNOL 7.2/1100 (Lithuania). After heat treatment the zeolite samples were cooled in a desiccator. All reagents used were of analytical grade. The 0.05% solution of Sulfarsazene was prepared using 0.05 M borax, all other reagent solutions using bidistilled water. The standard solution of Ag(I) (1.0 mg mL⁻¹) was obtained via dissolving AgNO₃ and the content of Ag(I) was determined titrimetrically using Moore's technique.

Antibacterial activities of high dispersed native (CN) and H-forms (CH) of clinoptilolite with Ag(I) (CN-Ag(I) and CH-Ag(I), respectively) were assayed using test cultures of gram-negative bacteria *Escherichia coli* ATCC 25922 and gram-positive bacteria *Staphylococcus aureus* ATCC 25923. Bacterial test cultures were obtained from the Culture collection of microorganisms – producers of antibiotics of Ivan Franko National University of Lviv (Lviv, Ukraine). The samples preparation of the natural form of clinoptilolite with Ag(I) composite was carried out under the optimal conditions of Ag(I) sorption on this zeolite as described in paper [3].

Adsorption measurements and methods of determination of silver content in the solutions. The sorption properties of H-clinoptilolite were studied under dynamic conditions in a SPE mode. Metal solutions were passed through a sorption cartridge filled with 0.6 g of the sorbent at a flow rate of 5 mL min⁻¹ using the peristaltic pump. The investigation techniques under dynamic conditions are described in detail in paper [35]. The passage moment of Ag(I) ions was determined by spectrophotometry

with Sulfarsazene. The absorbance of the complex solution was measured at 540 nm (DR/400 V spectrophotometer HACH) against the solutions which contained all components, except Ag(I). This method is a highly sensitive method for Ag(I) determination ($LDL = 100 \text{ ng mL}^{-1}$). For the desorption of Ag(I) from the zeolite bed, 15 mL of the eluent was passed through the sorption cartridge at a flow rate of 0.5 mL min^{-1} . The Ag(I) content in the eluates was determined by the flame version (propane – butane – air) of atomic absorption spectrophotometry using an AAS-1N atomic absorption spectrophotometer (Carl Zeiss, Jena) at $\lambda = 328.1 \text{ nm}$. The adsorption and desorption studies were carried out at a temperature of $20 \pm 1^\circ\text{C}$.

Determination of the antibacterial action of composites. The evaluation of antibacterial activities of the clinoptilolite composites was carried out as follows: CN, CH, CH-Ag(I) and CN-Ag(I) (0.1, 0.5 and 1.0 mg) were added to tubes with 0.9 mL of sterile distilled water, after that 0.1 mL of *E. coli* (titer $1.5 \times 10^{12} \text{ cells mL}^{-1}$) and *S. aureus* (titer $1.1 \times 10^{10} \text{ cells mL}^{-1}$) cells suspension was added to this mixture. CH-Ag(I) and CN-Ag(I) composites had a different concentration of Ag(I) (2.9, 5.2, 10.4 μg) which corresponded to its quantity in 1 mg of a composite; they were marked as CN-Ag2.9, CH-Ag2.9, CH-Ag5.2 and CN-Ag10.4. The aqueous suspension of test cultures without clinoptilolite composites was used as a control. The mixtures were shaken for 2 min and seeded with dilution (10^{-5} – 10^{-9}) on the L-agar media [36]. The number of colonies that survived was counted after 24 h. Fine dispersive samples (particles size $< 1 \mu\text{m}$) were used for the investigations.

Results and Discussion

Batch Adsorption Experiments

Effect of acid-modifier concentration. During the interaction of acids with natural zeolites the ionic exchange takes place, while the dealumination of these aluminosilicates is also possible. Obviously, the sorptive properties of acid-modified zeolites depend on concentration of acid used for modification, and also on the time of sorbent contact with acid solutions. That is why at the initial stage of the study the optimal conditions for preparation of acid-modified clinoptilolite samples exhibiting high sorption capacity towards Ag(I) were found. Nitric acid was used to modify the clinoptilolite. The sorption capacity of clinoptilolite samples modified with nitric acid solutions of various concentrations during 24 h was studied. According to [19], the contact of Transcarpathian clinoptilolite with acid solutions lasting for 24 h provides the maximal activity of the acid-modified form of this zeolite. It is notable from Fig.1, that the acid-modified sample obtained after the zeolite treatment with 1 M HNO_3 solution sorbs Ag(I) most efficiently.

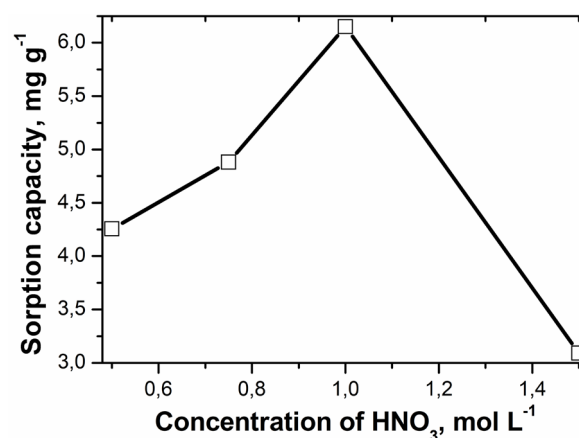


Fig. 1. Dependence of the sorption capacity of acid-modified clinoptilolite towards silver (I) on the nitric acid concentration as acid-modifier. The following conditions were used: Ag(I) concentration in solution = $1 \mu\text{g mL}^{-1}$; pH = 6.0; duration of modification, 24 h; solution flow rate through the sorbent = 5 mL min^{-1} ; diameter of the zeolite grains = (0.20–0.31) mm.

During the treatment of Transcarpathian clinoptilolite with diluted (0.1–1 M) acid solutions the ionic exchange mainly takes place without any significant dealumination of the zeolite [19]. The Transcarpathian clinoptilolite stands out among other natural zeolites due to the high acid resistance. That is why the common treatment with acid solutions can be used to obtain the H-form of this aluminosilicate. In our further investigations the H-clinoptilolite samples obtained using the 1 M HNO_3 solution were applied.

pH Effect. The sorption of Ag(I) on H-clinoptilolite depending on the medium acidity was studied (Fig. 2). The necessary pH values of Ag(I) solutions were supplied by adding the diluted solutions of NaOH or HNO_3 . The obtained results prove that Ag(I) is not sorbed on H-clinoptilolite in the strong acid medium. Starting from pH 3 the sorption of Ag(I) increases drastically and reaches its maximum in weakly acidic solutions at pH 6.0. It is known [19,23,26,27] that mainly the surface OH-groups are the sorption-active centers towards heavy metal ions in the acid-modified Transcarpathian clinoptilolite. That is why such way of Ag(I) sorption process can be caused by the chemical peculiarities of H-clinoptilolite surface and by existing forms of Ag(I) in aqueous solutions at different pH.

The dissociation of surface hydroxo-groups of the sorbent is almost completely depressed at low pH values, which is the reason for the small value of H-clinoptilolite sorption capacity towards Ag(I). While changing pH the transformation of Ag(I) existing form takes place. According to [37] Ag(I) exists in a form of $\text{Ag}(\text{H}_2\text{O})^+$ in weakly acidic solution at low concentrations ($\sim 10^{-5} \text{ M}$). This indicates that Ag(I) is sorbed on H-clinoptilolite basically according to the ion-exchange mechanism. In the alkaline solutions the anionic hydroxo-complex $[\text{Ag}(\text{OH})_2]^-$ is partly formed that is not sorbed by the zeolite.

Unlike the H-form of clinoptilolite, the maximal sorption capacity towards Ag(I) for the uncalcined samples of Transcarpathian clinoptilolite natural form is observed in weakly alkaline solutions at pH 8.0 and is equal to 1.73 mg g^{-1} [3]. As it can be seen from Fig.2, the maximal sorption capacity towards Ag(I) for the H-form of Transcarpathian clinoptilolite is equal to 6.15 mg g^{-1} , which is 3.5 times higher than the respective value for the sorption capacity of the uncalcined samples of this zeolite natural form.

As H-clinoptilolite sorbs Ag(I) efficiently from weakly acidic solutions, then the possibility of applying buffer solutions with pH 6.0 during the pre-concentration of Ag(I) trace amounts was investigated. It was indicated, that the sorption capacity of acid-modified clinoptilolite towards Ag(I) decreases significantly in the medium of phosphate, universal, acetic, citric, urotropine and phthalate buffer solutions. The highest value of H-clinoptilolite sorption capacity is observed in the medium of phthalate buffer solution and it is equal to only 0.425 mg g^{-1} , which is a 14.5 times lower value than the sorption capacity of this sorbent in solutions without buffers. So, the application of buffer solutions during the pre-concentration of Ag(I) on H-clinoptilolite is practically impossible.

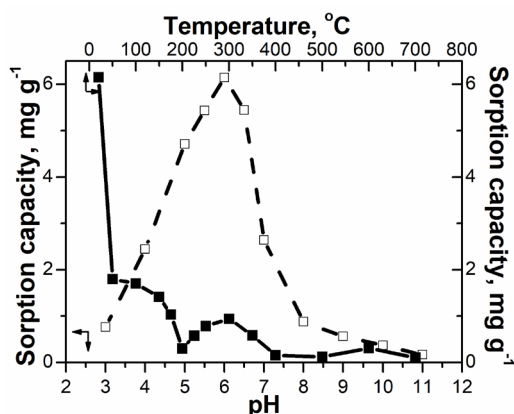


Fig.2. Dependence of acid-modified clinoptilolite sorption capacity of silver(I) on a pH value of the aqueous solution and thermal treatment carried out in the range from 20 to 700°C (concentration of Ag(I) – $1 \mu\text{g mL}^{-1}$; pH = 6.0; flow rate – 5 mL min^{-1} ; time of heat treatment, 2.5 h); diameter of the zeolite grains = (0.20–0.31) mm.

Grain Fraction Effect. The dependence of the dynamicsorption capacity of acid-modified-clinoptilolite towards Ag(I) on the degree of zeolite dispersion was investigated (Table 2). The results are listed in Table 2 showing that the sorption capacity of H-clinoptilolite towards Ag⁺ ions increased negligibly as the sorbent grains size decreased. This means that in a dynamic regime it is recommended to employ zeolites with the smallest size of grains. Nevertheless, it was found that the use of granules within dimensions less than 0.20–0.31 mm diameter caused experimental difficulties since such highly dispersed sorbents generated a high

resistance to solution flow under dynamic conditions. For this reason, acid-modified-clinoptilolite granules of 0.20–0.31 mm dimensions were chosen as the optimum in subsequent experiments.

Table 1. Values of H-clinoptilolite sorption capacity towards Ag(I) in the medium of different buffer solutions (concentration of Ag(I) = $1.0 \mu\text{g mL}^{-1}$; pH 6.0).

Buffer solution	Concentration of the buffer solution, mol L^{-1}	Sorption capacity, mg g^{-1}
Phosphate	1.3×10^{-4}	0.31
Universal	2.0×10^{-3}	0.15
Acetic	7.0×10^{-3}	0.115
Citric	1.0×10^{-3}	0.23
Urotropine		0.215
Phthalate	6.0×10^{-4}	0.425
Bufferless solution	–	6.15

Table 2. Dependence of the sorption capacity of H-clinoptilolite towards silver(I) on the grain size of the sorbent particles (concentration of Ag(I) = $1.0 \mu\text{g mL}^{-1}$; pH 6.0).

Particle fraction size, mm	Sorption capacity, mg g^{-1}
0.20–0.31	6.15
0.31–0.35	5.74
0.35–0.50	5.30
0.50–0.70	5.24

Effect of the Precalcination Temperature of H-Clinoptilolite. Sorptive properties of acid-modified Transcarpathian clinoptilolite depend on its preliminary thermal treatment [19,23,26]. For this reason the H-clinoptilolite samples were heated at different temperatures for 2.5 h, and after cooling in a desiccator their sorption capacity towards Ag(I) was determined. The results obtained are illustrated on Fig.2. As it can be seen from Fig.2, the uncalcined H-clinoptilolite samples sorb Ag(I) most efficiently. In a low temperature range below 200°C the decrease of sorption efficiency is observed on the H-clinoptilolite sorption efficiency dependence on the temperature of sorbent preliminary thermal treatment. Especially considerable decrease of sorption capacity towards Ag(I) is observed in the acid-modified clinoptilolite samples pre-heated at 50°C . In the Transcarpathian clinoptilolite at the temperature of $<100^{\circ}\text{C}$ the removal (evaporation) of the liquid water surface layer takes place [38]. So, possibly, exactly the evaporation of the liquid water surface layer causes the significant decrease of sorption efficiency towards Ag(I) of H-clinoptilolite samples pre-heated at 50°C . The insignificant decrease of acid-modified clinoptilolite sorption capacity in the temperature range $50 - 200^{\circ}\text{C}$ is probably due to the partial structure amorphization of H-clinoptilolite, which takes place in this temperature range [19].

H-clinoptilolite samples preliminarily calcined at temperatures >200 °C exhibit low sorption efficiency towards Ag(I). On the curve of Fig. 2 two small maxima are registered in a high temperature range. Such temperature values are characteristic for acid-modified clinoptilolite. In particular, on the curve describing the dependence of H-clinoptilolite sorption capacity towards Cd(II) [19] and Pb(II) [23] on temperature of preliminary thermal treatment of this sorbent the maxima of different intensity are also observed in the temperature range of 300 and 600 °C.

Authors of papers [39,40] provided a detailed description of the transformation processes during the calcination of clinoptilolite's H-form at different temperatures. Particularly, it was shown that the H-form of clinoptilolite partially undergoes successive changes at temperatures of 300, 400 and 600 °C according to the Equations 1 and 2.

The process of dealumination is followed by a decrease in intersurface distances and can be attributed to the formation of siloxan bonds at the places of tetrahedral vacancies, according to the following reaction mechanism (Equation 3).

In the acid-modified Transcarpathian clinoptilolite mainly the surface OH-groups are the sorption active centers towards heavy metal ions [19,23,26]. Diagram 1 suggests that at a temperature of 300 °C a partial transformation of H-clinoptilolite takes place accompanied with the quantity increase of OH-groups within the structure of the zeolite. Obviously, a slight increase in the efficiency of adsorption of H-clinoptilolite samples calcined at 300 °C (Fig. 2) is connected with the increase of the number of surface OH-groups according to the Equation 1.

The decrease in the sorption capacity of acid-modified clinoptilolite annealed at ≥400 °C can be attributed to the acceleration of the process of zeolite amorphization (Diagram 2). X-ray investigations have also proved that in the temperature range of 350–450 °C the amorphization of H-clinoptilolite is most drastic and the unit cell volume at 400 °C is minimal [19].

The Si–O–Si group is known as one of the clinoptilolite functional groups that heavy metals

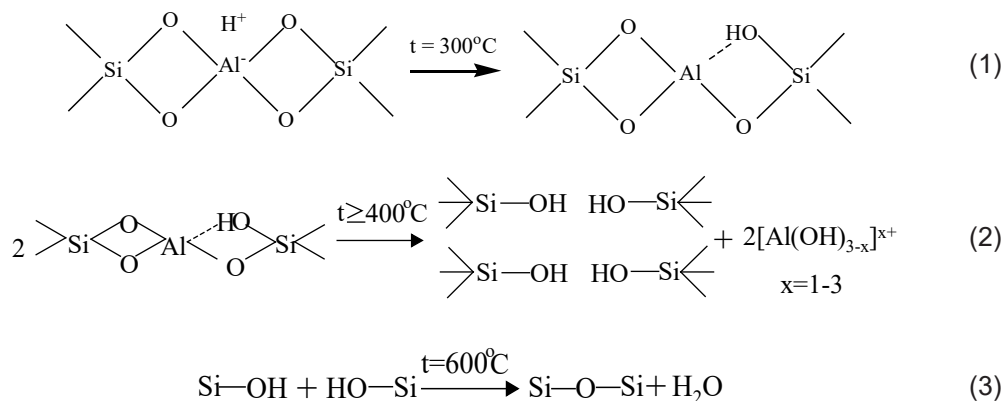
interact with [41]. The negligible increase of the sorption capacity of acid-modified-clinoptilolite heated at the temperature close to 600 °C (Fig. 2) may be attributed to the new formed siloxan bonds (Equation 3) on tetrahedral vacancies which were formed during the process of dealumination of H-clinoptilolite according to Equation 2. The minimal sorption capacity value of samples calcined at the temperature of 700 °C is caused by almost complete amorphization of the zeolite [19].

Desorption. In order to find efficient desorbents of Ag(I) the solutions of HNO₃, NH₄OH and also nitrates of calcium and alkali metals were tested. The desorption results (Table 3) assure that solutions of HNO₃, NaNO₃, CsNO₃ and RbNO₃ are the efficient desorbents. Solutions of HNO₃ provided a 100% desorption of silver pre-concentrated on H-clinoptilolite.

Table 3. Desorption efficiency of silver (I) from acid-modified clinoptilolite^a.

Desorbent	Desorption (%)
7 M HNO ₃	100
3.5 M HNO ₃	100
1 M NaNO ₃ (pH 4.0)	88
0.1 M NaNO ₃ (pH 4.0)	60
0.1 M NaNO ₃	60
1 M CsNO ₃ (pH 4.0)	70
0.1 M CsNO ₃ (pH 4.0)	80
0.1 M CsNO ₃	65
1 M RbNO ₃ (pH 4.0)	70
0.1 M RbNO ₃ (pH 4.0)	75
0.1 M RbNO ₃	35
0.1 M KNO ₃ (pH 4.0)	35
0.1 M KNO ₃	25
6.6 M NH ₄ OH	20
0.1 M Ca(NO ₃) ₂ (pH 4.0)	8
0.1 M Ca(NO ₃) ₂	8

^aFlow rate of an eluent through the adsorption system = 0.5 mL min⁻¹; volume of the eluent employed = 15 mL; the acidification of salts solutions to a pH value of pH 4.0 was carried out using the solution of HNO₃.



Influence of foreign ions on Ag(I) sorption. The influence of common ions of waters on the preconcentration of Ag(I) with acid-modified clinoptilolite from the solutions was studied at pH 6.0. The concentration of a foreign ion, at which the decrease of the H-clinoptilolite sorption capacity towards Ag(I) was not observed, was considered as tolerable. As it can be seen from Table 4, the (10–100)-fold excesses of common components of waters do not affect the sorption of Ag(I) trace amounts. In the proposed method of Ag(I) preconcentration the sorption capacity of H-clinoptilolite is used that does not exceed 10 % of the maximal sorption capacity. This means, that if foreign species at high concentrations slightly decrease the maximal sorption capacity, this has no influence on the preconcentration of Ag(I) trace amounts. That is why the values of allowed excesses of foreign ion contents are usually significantly higher in comparison with the allowed excesses of these ions' contents that lead to the decrease of maximal sorption capacity when they are exceeded. It is known that beside Ag(I) the acid-modified Transcarpathian clinoptilolite sorbs some other heavy metals, in particular Cd(II) [19], Pb(II) [23], Sc(III) [26], Pd(II) [27]. However the optimal sorption conditions are different for various metals. Ag(I) cations are most effectively sorbed by H-clinoptilolite from the weakly acidic solutions at pH 6.0 according to ion-exchange mechanism (Figure 2). At the same time Pb(II), Sc(III) and Ni(II) are practically not sorbed from weakly acidic solutions. The sorption capacity of H-clinoptilolite towards Cd(II) and Pb(II) in solutions with pH 6.0 is also low [19,27]. So, the selectivity of Ag(I) preconcentration is mainly achieved by means of different optimal sorption conditions for Ag(I) and other heavy metals. Additionally the selectivity of Ag(I) preconcentration could be enhanced even at the desorption stage. The case is that using HNO₃ solutions the desorption of 100% Ag(I) from the H-clinoptilolite matrix is achieved (Table 3). At the same time the desorption efficiency of Cd(II), Pb(II) and Sc(III) by means of HNO₃ solutions is much lower [19,23,26]. That is why the relative content of Ag(I) in eluates obtained during the desorption will increase.

Natural zeolites and their H-forms can sorb organic substances that are in a form of cation or neutral organic molecules. Anionic forms of organic substances practically are not sorbed on these sorbents. The selectivity of Ag(I) preconcentration in the presence of organic substances is provided during the desorption stage. As it can be seen from Table 3, the effective desorbents of Ag(I) from H-clinoptilolite matrix are solutions of HNO₃ and alkali metal salts. At the same time these desorbents are not efficient for organic substances at all. Organic substances are usually desorbed from zeolites using different organic solvents [30-32]. That is why the eluates obtained during the desorption of Ag(I) practically do not contain organic substances.

Table 4. Tolerance limits of some ions for silver(I) sorption from aqueous solution of H-clinoptilolite (concentration of Ag(I) = 1 µg mL⁻¹; pH 6.0).

Species	Tolerance limit ($C_{ion}/C_{Ag(I)}$)
Fe ³⁺ , NO ₃ ⁻ , CO ₃ ²⁻	100
Na ⁺ , NH ₄ ⁺	75
K ⁺	20
Mg ²⁺	15
Ca ²⁺	10

Basing on the generalization of the investigations results such optimal conditions for Ag(I) sorption on H-clinoptilolite were proposed: the zeolite modifier – 1 M solution of nitric acid; the grain diameter of sorbent – 0.20–0.31 mm; pH 6.0; flow rate of Ag(I) solution with the concentration of 1 µg mL⁻¹ through the sorbent – 5 mLmin⁻¹. The maximal value of the acid-modified Transcarpathian clinoptilolite sorption capacity towards Ag(I) at the optimal conditions is equal to 6.15 mg g⁻¹.

Analytical performance

High sorption capacity of the acid-modified Transcarpathian clinoptilolite, its ability to sorb low concentrations of Ag(I), the presence of efficient desorbents, the possibility of Ag(I) sorption on the background of water common components give the reason to suggest this modified natural zeolite for the preconcentration of Ag(I) at the stage of water samples preparation for the analysis.

The method of trace amounts of Ag(I) preconcentration in a solid phase extraction mode during the atomic absorption analysis of waters has been proposed.

Sample preconcentration procedure. The sorbent was prepared the following way: the natural Transcarpathian clinoptilolite sample was grained in a ball-mill; a zeolite fraction with a 0.20–0.30 mm grain size was taken, washed with bidistilled water and dried at 20 °C. The dried clinoptilolite sample (6 g) was treated with 0.2 L of 1 M HNO₃ solution for 24 h. After that the zeolite was washed from all possible anions with bidistilled water and dried at 20 °C. The solution of HNO₃ was added to 0.5–2.5 L of the investigated water to pH~1 and the solution was heated on the sand bath for 1 h. The mixture was filtered through the dense paper filter, the pH of the solution was adjusted to 6.0 with a NaOH solution. The obtained mixture was passed through a SPE cartridge containing 0.6 g of the prepared sorbent at a flow rate of 3 mL min⁻¹. After the sample being loaded, the cartridge was washed with 50 mL of bidistilled water at the same flow rate. Whereas Ag(I) ions were desorbed with 15 mL of a 3.5 M nitric acid solution at a flow rate 0.5 mLmin⁻¹. Silver content in the solution was determined by the atomic absorption method, as described in detail in the Section 2 "Materials and Methods". In general, the proposed method for determination of Ag(I) ions had a

linearity range from 0.95 to 100 ng mL⁻¹. The detection limit was found to be 0.3 ng mL⁻¹, and this parameter was calculated using the following Equation:

$$DL = 3S_b/m, \quad (4)$$

where S_b is a standard deviation of a blank and m is a slope of the calibration curve.

The method was tested using a tap water, and also water from the lake of Pischne in the Shatsky National Natural Park (Western Ukraine). In some literature sources it is only mentioned about the high silver content in the water of the Pischne lake without providing the exact concentration of this physiologically active component of waters. The qualitative spectral analysis of sapropele mules from this lake to estimate the microelements content was carried out by authors [42]. Among 28 rare and scattered chemical elements found in the investigated sapropele mules there was also silver. The results of tap water analysis using the standard addition method prove, that the matrix effect is completely eliminated (Table 5). Unlike the investigated tap water, the water from Lakes of Shatsk is characterized with a low mineralization and rigidity. In particular, the overall mineralization of water from the Pischne lake is equal to 132 mgL⁻¹, and the concentration of Ca²⁺ and Mg²⁺ is equal to only 19 and 3 mgL⁻¹, respectively, that is why obviously the ionic constitution of the investigated lake water also cannot have influence on the preconcentration of Ag(I) trace amounts. The analyte recoveries from spiked tap water were above 98–100 %.

The concentration of silver in the water of the Pischne lake is equal to 17 ng mL⁻¹. Such silver content is considered as increased for natural surface waters.

Antibacterial properties of the H-clinoptilolite and clinoptilolite composites with silver. Two forms CH and CN of clinoptilolite and their composites with Ag(I) were tested against gram-negative and gram-positive bacteria. We found that CN with the concentrations of 0.1, 0.5 and 1.0 mg mL⁻¹ suppresses survival of *E. coli* to 6.3±0.9, 0.6±0.1 and 0.2±0.05 %, respectively (Fig. 3).

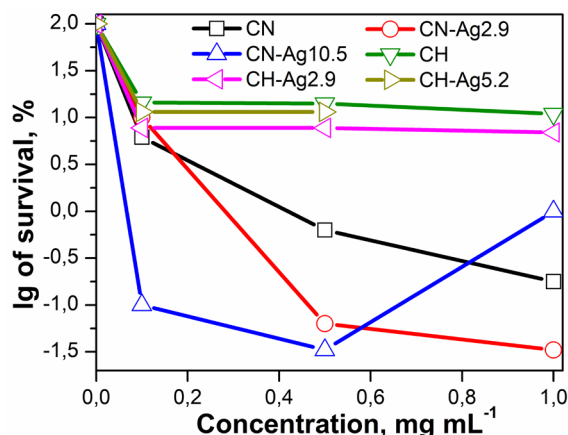


Fig. 3. The impact of different forms of clinoptilolite-Ag(I) composite on the survival of *E. coli*. For axis of abscissas – concentration, mg mL⁻¹; for axis of ordinates – lg % of survival.

The increase of CN-Ag2.9 concentration to 0.5 mg mL⁻¹ led to a drastic decrease of *E. coli* survival (0.06±0.01 %). However, the increase of CN-Ag2.9 concentration to 1 mg mL⁻¹ did not significantly reduce the survival of *E. coli* cells. The composite of clinoptilolite with higher silver concentration of 0.1 mg mL⁻¹ (CN-Ag10.5) increased the death rate of *E. coli* cells in the suspension by an order of magnitude. However, after increasing the composite concentration to 0.5 mg mL⁻¹ the reduction of bacteria survival became insignificant.

CH in the concentrations used decreased the survival of *E. coli* to 11.2±1.9 – 14.6±2.0 %, which is 2-100 less effective than CN. The composition of CH-Ag 2.9 caused a 2-fold decrease of *E. coli* survival (until 7.0±0.8 – 7.9±0.9 %) compared with CH. The increase of silver concentration to a value of 0.5 mg mL⁻¹ (CH-Ag 5.2) did not contribute to the enhancement of antibacterial activity against *E. coli*.

Table 5. The results of FAAS determination of silver(I) in the tap water and water from the lake of Pischne after the solid phase preconcentration using H-clinoptilolite (n = 3; P = 0.95).

Volume of water sample (mL)	Enrichment factor ^b	Concentration of Ag(I) (ng mL ⁻¹)		Recovery (%)	RSD (%)
		Added	Found		
Tap water					
600	40	50	49±3	98	2.5
1500	100	35	35±2	100	2.3
2400	160	0	ND ^c		
Water from the lake Pischne					
1500	100	0	17±2		4.7

RSD: relative standard deviation. ^athe water sample from the lake of Pischne was taken on August 23rd, 2018.

^bEnrichment factor = volume of sample / volume of eluent. ^cND < detection limit.

The effect of different CN concentration on the survival of *S. aureus* was weak and amounted to $60.0 \pm 5.7 - 62.5 \pm 6.3\%$ (Fig.4). The antagonistic activity of the CH against *S. aureus* was similar to the antagonistic activity of the CN. But in their composites with silver CH-Ag(I) ($0.1 - 1.0 \text{ mg mL}^{-1}$) the survival of *S. aureus* decreased to $40.5 \pm 3.9 - 48.0 \pm 5.8\%$ (CH-Ag 2.9) and $23.8 \pm 2.5 - 29.8 \pm 2.9\%$ (CH-Ag 5.2).

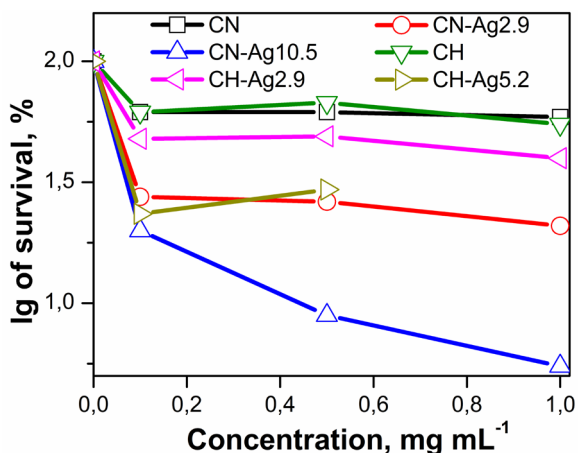


Fig.4. The impact of different forms of clinoptilolite-Ag(I) composite on the survival of *S. aureus*. For axis of abscissas – concentration, mg mL^{-1} ; for axis of ordinates – lg % of survival.

Therefore, the natural form of clinoptilolite has a better antibacterial effect against *E. coli* than the H-form. However, antagonistic activity against *S. aureus* was worse than against *E. coli* in both cases. The combination of different clinoptilolite forms with Ag(I) increases their antibacterial activity. However, the composition of CN form with different concentrations of Ag(I) exhibited more powerful antibacterial effect than the CH-Ag(I) composite.

Among 30 natural zeolites only clinoptilolite is allowed to be applied in the medical practice. That is why in perspective the composites of the natural and H-form of Transcarpathian clinoptilolite with silver can be used as medical and veterinary antimicrobial remedies for an external use. On applying such remedy the leak of toxic Ag^+ into the biosubstrates becomes

References

1. Kagaya S.; Maeba E.; Inoue Y.; et al. A solid phase extraction using a chelate resin immobilizing carboxymethylated pentaethylenhexamine for separation and preconcentration of trace elements in water samples. *Talanta* **2009**, 79, 146–152. <https://doi.org/10.1016/j.talanta.2009.03.016>
2. Ayata S.; Kaynak I.; Merdivan M. Solid phase extractive preconcentration of silver from aqueous samples. *Environ. Monit. Assess.* **2009**, 153, 333–338. <https://doi.org/10.1007/s10661-008-0359-6>
3. Vasylechko V.O.; Fedorenko V.O.; Gromyko O.M.; et al. Solid phase extractive preconcentration

practically impossible because the desorption of silver from the composites Ag-H-clinoptilolite and Ag-clinoptilolite does not take place at such conditions.

Conclusions

The sorption properties of acid-modified Transcarpathian clinoptilolite towards Ag(I) ions in the aqueous solution using the dynamic technique were investigated. It was established that Ag(I) is recovered with the highest efficiency from the moderately acidic solutions mainly according to the ion-exchange mechanism. In general, the optimal conditions for Ag(I) sorption on H-clinoptilolite are the following: zeolite grains size – $0.20-0.31 \text{ mm}$, Ag(I) solution flow rate – 5 mL min^{-1} , pH 6.0. It was found that the most effective acid-modifier is 1 M solution of nitric acid. The maximal adsorption capacity of acid-modified clinoptilolite with respect to Ag(I) ions of 6.15 mg g^{-1} can be achieved in this case.

3.5 M solutions of the nitric acid were found to be the best eluents of silver from acid-modified clinoptilolite. The developed SPE technique offers a possibility of Ag(I) trace amounts preconcentration in the presence of other components of water before FAAS determination. An enrichment factor of 160 was obtained under the optimal conditions. A wide range of linearity ($0.95-100 \text{ ng mL}^{-1}$) with the detection limit of 0.3 ng mL^{-1} was achieved. The developed procedure was applied for the determination of Ag(I) ions in the tap and lake water, whereas the recoveries and RSD values were 98-100 and 2.3–4.7 %, respectively.

It was determined, that the native form of clinoptilolite has a better antibacterial effect against *E. coli* than its H-form. However, antagonistic activity against *S. aureus* was worse than against *E. coli* in both forms. Combining of different forms of clinoptilolite with Ag(I) increases their antibacterial activity.

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Conflicts of Interest

The authors declare no conflict of interest.

of silver from aqueous samples and antimicrobial properties of the clinoptilolite–Ag composite. *Adsorp. Sci. Technol.* **2017**, 35 (7–8), 602–611. <https://doi.org/10.1177/0263617417703509> journals.sagepub.com/home/adt

4. Vasylechko V.; Fedorenko V.; Gromyko O.; et al. A novel solid-phase extraction method for preconcentration of silver and antimicrobial properties of the Na-clinoptilolite–Ag composite. In Press: *Materials today: proceedings 2019* <https://doi.org/10.1016/j.matpr.2019.10.049>

5. Zolotov Yu.A.; Tsyzin G.I.; Morosanova E.I.; et al. Sorption preconcentration of trace components for

chemical analysis. *Uspekhi Khimii* **2005**, 74 (1), 41–66.

6. Fisher A.; Kara D. Determination of rare earth elements in natural water samples – A review of sample separation, preconcentration and direct methodologies. *Anal. Chim. Acta* **2016**, 74 (1), 1–29. <https://doi.org/10.1016/j.aca.2016.05.052>

7. Tan X.; Ren X.; Chen C.; et al. Analytical approaches to the speciation of lanthanides at solid-water interfaces. *Trends Anal. Chem.* **2014**, 61, 107–132. <https://doi.org/10.1016/j.trac.2014.06.010>

8. Zougagh M.; Pavon J.M.C.; de Torres A.G. Chelating sorbents based on silica gel and their application in atomic spectrometry. *Anal. Bioanal. Chem.* **2005**, 381, 1103–1113. <https://doi.org/10.1007/s00216-004-3022-2>

9. Zaporozhets O.A.; Zinko L.S.; Keda T.Ye.; et al. Solid-phase spectrophotometric determination of nickel and zinc using dithizone immobilized. *Methods Objects Chem. Anal.* **2007**, 2 (1), 62–69. <http://www.moca.net.ua/07/pdf/02012007-062.pdf>

10. Ahmadi M.; Elmongy H.; Madrakian T.; et al. Nanomaterials as sorbents for sample preparation in bioanalysis: A review. *Anal. Chim. Acta* **2017**, 958, 1–21. <https://doi.org/10.1016/j.aca.2016.11.062>

11. Kostenko L. S.; Tomashchuk I. I.; Kovalchuk T. V.; et al. Bentonites with immobilized Organophosphorus Complexing Ligands as Adsorbent for the Removal of Toxic Metals from Natural water. *Methods Objects Chem. Anal.* **2018**, 13 (1), 35–43.

12. Faghihian H.; Kabiri-Tadi M. A novel solid-phase extraction method for separation and preconcentration of zirconium. *Microchim. Acta* **2010**, 168, 147–152. <https://doi.org/10.1007/s00604-009-0273-9>.

13. Ezzeddine Z.; Batonneau-Gener I.; Pouilloux Ya.; et al. Synthetic Nax Zeolite as a Very Efficient Heavy Metals Sorbent in Batch and Dynamic Conditions. *Colloids Interfaces* **2018**, 2, 22–35. <https://doi.org/10.3390/colloids2020022>.

14. Lihareva N.; Dimova L.; Petrov O.; et al. Ag⁺ sorption on natural and Na-exchanged clinoptilolite from Eastern Rhodopes, Bulgaria. *Micropor. Mesopor. Mater.* **2010**, 130, 32–37. <https://doi.org/10.1016/j.micromeso.2009.10.009>

15. Concepción-Rosabal B.; Rodríguez-Fuentes G.; Bogdanchikova N.; et al. Comparative study of natural and synthetic clinoptilolites containing silver in different states. *Micropor. Mesopor. Mater.* **2005**, 86, 249–255. <https://doi.org/10.1016/j.micromeso.2005.07.027>

16. Top A.; Ülkii S. Silver; zinc; and copper exchange in a Na-clinoptilolite and resulting effect on antibacterial activity. *Appl. Clay Sci.* **2004**, 27, 13–19. <https://doi.org/10.1016/j.clay.2003.12.002>

17. Godelitsas A.; Armbruster T. HEU-type zeolites modified by transition elements and lead. *Micropor. Mesopor. Mater.* **2003**, 61, 3–24. [https://doi.org/10.1016/s1387-1811\(03\)00352-4](https://doi.org/10.1016/s1387-1811(03)00352-4)

18. Dimova L.; Petrov J.; Kadiyski M.; et al. Preparation and Rietveld refinement of Ag-exchanged

clinoptilolite. *Clay Miner.* **2011**, 46, 205–212. <https://doi.org/10.1180/claymin.2011.046.2.205>

19. Vasylechko V. O.; Gryshchouk G. V.; Kuz'ma Yu. B.; et al. Adsorption of cadmium on acid-modified Transcarpathian clinoptilolite. *Micropor. Mesopor. Mater.* **2003**, 60, 183–196. [https://doi.org/10.1016/s1387-1811\(03\)00376-7](https://doi.org/10.1016/s1387-1811(03)00376-7)

20. Vasylechko V. O.; Gryshchouk G. V.; Zakordonskiy V. P.; et al. Sorption of terbium on Transcarpathian clinoptilolite. *Micropor. Mesopor. Mater.* **2013**, 167, 155–161. <https://doi.org/10.1016/j.micromeso.2012.08.021>

21. Vasylechko V. O.; Gryshchouk G. V.; Zakordonskiy V. P.; et al. A solid-phase extraction method using Transcarpathian clinoptilolite for preconcentration of trace amounts of terbium in water samples. *Chem. Cent. J.* **2015**, 9, 45–51. <https://doi.org/10.1186/s113065-015-0118-z>

22. Vasylechko V. O.; Gryshchouk G. V.; Zakordonskiy V. P.; et al. Sorption-luminescence method for determination of terbium using Transcarpathian clinoptilolite. *Talanta* **2017**, 174, 486–492. <https://doi.org/10.1016/j.talanta.2017.06.052>

23. Vasylechko V. O.; Gryshchouk G. V.; Kaminska M. I.; et al. A solid-phase extraction method using acid-modified Transcarpathian clinoptilolite for preconcentration of trace amounts of lead in water samples. *Appl. Nanosci.* **2019**, 9 (5), 1057–1065. <https://doi.org/10.1007/s13204-018-0858-x>.

24. Mamba B.B.; Nyembe D.W.; Mulaba-Bafubiandi A.F. Removal of copper and cobalt from aqueous solutions using natural clinoptilolite. *Water SA* **2009**, 35 (3), 307–314. <http://www.scielo.org.za/pdf/wsa/v35n3/a09v35n3.pdf>

25. Rakitskaya T.L.; Raskola L.A.; Kiose T.A.; et al. Adsorption of 3d metal ions by natural and acid-modified clinoptilolite. *Visn. Odes. nac. univ., Him.* **2010**, 15 (3), 85–91. <http://heraldchem.onu.edu.ua/article/view/43826/40135>

26. Vasylechko V.O.; Korpalo Ch.B.; Gryshchouk G.V. Acid-Modified Clinoptilolite – Effective Sorbent of Sc(III) from Aqueous Solutions. *Solid State Phenom.* **2015**, 230, 8–13. <https://doi.org/10.4028/www.scientific.net/SSP.230.8>

27. Korkuna O.; Vrublevska T.; Reschetilowski W. Study of Pd (II) Sorption from Aqueous Solutions on the Natural and Acidic-Modified Transcarpathian Clinoptilolite. *Polish J. Chem.* **2008**, 82, 431–442.

28. Singh V.V.; Jurado-Sánchez B.; Sattayasamitsathit S.; et al. Multifunctional Silver-Exchanged Zeolite Micromotors for Catalytic Detoxification of Chemical and Biological Threats. *Adv. Funct. Mater.* **2015**, 25 (14), 2147–2155. <https://doi.org/10.1002/adfm.201500033>

29. Jang Y.J.; Kim K.; Tsay O.G.; et al. Destruction and Detection of Chemical Warfare Agents. *Chem. Rev.* **2015**, 115 (24), PR1–PR76. <https://doi.org/10.1021/acs.chemrev.5b00402>.

30. Mohseni S.N.; Amooey A.A.; Tashakkorian

- H.; Amoue A.I. Removal of dexamethasone from aqueous solutions using modified clinoptilolite zeolite (equilibrium and kinetic). *Int. J. Environ. Sci. Technol.* **2016**, 13(9), 2261–2268. <https://doi.org/10.1007/s13762-016-1045-9>
31. Halkevych I.; Ivanauskas L.; Barsteigiene Z. High performance liquid chromatographic determination of bupropion using H-clinoptilolite as a sorbent for plasma and urine purification. *Chem. Chem. Technol.* **2015**, 9(2), 245–249. http://ena.lp.edu.ua/bitstream/ntb/28207/1/020_117_122.pdf
32. Halkevych I.Yo.; Zimenkovsky B.S. Determination of venlafaxine in blood plasma by high performance liquid chromatography after purification on H-clinoptilolite columns. *Clinical Pharmacy, Pharmacotherapy & Medical Standardization.* **2014**, 1-2, 109–112. http://nbuv.gov.ua/UJRN/Kff_2014_1-2_20.
33. Vasylechko V.O.; Gryshchouk G.V.; Lebedynets L.O.; et al. Adsorption of Copper on Transcarpathian Clinoptilolite. *Adsorpt. Sci. Technol.* **1999**, 17 (2), 125–134. <https://doi.org/10.1177/026361749901700206>
34. Tarasevich Y.I.; Polyakov V.E.; Penchev V.Zh.; et al. Ion-exchange qualities and structural features of clinoptilolites of various deposits. *Khim. Technol. Vody* **1991**, 13 (2), 132–140.
35. Vasylechko V.O.; Lebedynets L.O.; Gryshchouk G.V.; et al. Adsorption of Copper on Transcarpathian Mordenite. *Adsorpt. Sci. Technol.* **1996**, 14 (5), 267–277. <https://doi.org/10.1177/026361749601400501>
36. Kieser T.; Bibb M.; Buttner M.; et al. (2000) *Practical Streptomyces Genetics*, John Innes Foundation, Norwich, **2000**. <https://www.jic.ac.uk/science/molmicro/Strepmanual/Manual.htm>
37. Baes C.F.; Mesmer R.E. *The Hydrolysis of Cations*, John Wiley & Sons, New York – London – Sidney – Toronto, **1976**. <https://doi.org/10.1002/bbpc.19770810252>
38. Zakordonski V.P.; Vasylechko V.O.; Staszczuk P.; et al. Water thermodesorption and adsorption properties of the transcarpathian zeolites. *Visnyk Lviv. Univ. Ser. Khim.* **2004**, 44, 247–256. <https://doi.org/http://ua.convdocs.org/docs/index-239615.html>
39. Tomazović B.; Čeranic T.; Sijarić G. The properties of the NH₄-clinoptilolite. Part 1. *Zeolites* **1996**, 16, 301–308. [https://doi.org/10.1016/0144-2449\(95\)00118-2](https://doi.org/10.1016/0144-2449(95)00118-2)
40. Tomazović B.; Čeranic T.; Sijarić G. The properties of the NH₄-clinoptilolite. Part 2. *Zeolites* **1996**, 16, 309–312. [https://doi.org/10.1016/0144-2449\(95\)00117-4](https://doi.org/10.1016/0144-2449(95)00117-4)
41. Argun M.E. Use of clinoptilolite for the removal of nickel ions from water: Kinetics and thermodynamics. *J. Hazard. Mater.* **2008**, 150, 587–595. <https://doi.org/10.1016/j.jhazmat.2007.05.008>
42. Khmelivsky V.O.; Baranov V.I.; Kostyuk O.V.; Sapropelle muds from lakes of Shatsk national park. *Nature Reserves in Ukraine*, **2011**, 17 (1), 94–96. <http://aetos.kiev.ua/zsu/zsu17/zsu17-16.pdf>