ANTIBACTERIAL ACTIVITY OF METAL-LOADED ZEOLITES AGAINST *ESCHERICHIA COLI*

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ABSTRACT
The antibacterial activity of metal-modified natural clinoptilolite (CLI) and zeolite A (metal - Cu^{2+}, Zn^{2+}, Ag^{+}) were tested against wildtype *Escherichia coli* strain DSM 498. Real water from Sava Lake (Belgrade) was used in the experiments. The zeolite samples contained similar amount of metal (0.25 mmol/g zeolite) but, exhibited different degree of antibacterial activity. The antibacterial activity of metal-loaded zeolites depends on the zeolite concentration, type of zeolite, time of contact and metal nature. After 1 h of contact only Ag-CLI and Ag-A exhibits the bactericidal effect, but after 24 h the bactericidal activity was found for all the examined zeolite systems.

Keywords: clinoptilolite, zeolite A, copper, silver, zinc, antibacterial activity.

INTRODUCTION
In the last decade, many inorganic compounds with antimicrobial properties have been reported. Among them, compounds containing heavy metal ions such as silver, copper or zinc are of interest because of their strong antimicrobial activity, chemical stability, and a wide antimicrobial spectrum. Zeolites have been frequently used as the host material for the preparation of antimicrobial materials. Recent studies indicate that particle of metal-containing zeolites acts bactericidal itself, without the need to release metal ions. [1, 2]
Escherichia coli is a Gram-negative, facultative anaerobic, non spore forming bacterium from the family *Enterobacteriaceae*. *E. coli* is normal habitant of human and animal colon and therefore is used as an indicator of faecal contamination of water, soil or food. Some strains of *E. coli* are human and animal pathogens.
Here we reported the antibacterial activity of Cu^{2+}-, Ag^{+}-, Zn^{2+}-containing natural clinoptilolite and zeolite A against *E. coli* DSM 498.

EXPERIMENTAL
Natural zeolite used in the experiments was obtained from Iran (Semnan) and zeolite A was from Linde Company (LTA, 4A). The metal-loaded zeolites were prepared by an ion-exchange procedure mixing the zeolite and corresponding metal solution in a weight ratio of 1:100, shaking suspension at 25-45 °C in a thermostatic water bath (Memmert WNB22). After 24 h the suspensions were separated by filtration and the products were dried at the 60 °C over night. The experimental conditions are given in Table 1.

Table 1. Experimental conditions for the preparation of the metal-containing zeolite samples

	Metal	Concentration of the solution [ppm]	Temperature [°C]	Samples
A	Ag	300	25	Ag-A
	Cu	200	45	Cu-A
	Zn	200	45	Zn-A
CLI	Ag	400	25	Ag-CLI
	Cu	400	45	Cu-CLI
	Zn	400	45	Zn-CLI

Dry samples were sterilized by autoclaving (121º C, 30 min) before testing the antibacterial activity. Ag-A and Ag-CLI were sterilized at 70 ºC for 1 h in a dry sterilizator (Binder B28, Germany). No microbial contamination of the prepared zeolite samples was found.

The fresh sample of real water from Sava Lake was firstly filtrated through the Buchner funnel with a filter paper (blue band) and then through the glass funnel B4. *E. coli* strain DSM 498 was used. Bacterial biomass was firstly pre-grown on a nutrient agar (Torlak, Serbia) for 16 h at 37±0.1º C to obtain the cultures in a log phase of growth. Then, it was suspended in 0.85 wt. % NaCl and mixed by a vortex. The prepared biomass (1 cm^3) was inoculated into the Schott's bottles with 100 cm^3 of autoclaved Sava lake water and the bottles were incubated in a thermostatic water bath (Memmert WNB22) during 24 h at 37±0.1º C with shaking at 105 rpm. As a control system, the Schott's bottles with parent zeolites at concentration of 1 g/100 cm^3 was set up. The metal-containing zeolites were added in different concentrations (0.1; 0.5 and 1.0 g/100 cm^3).

The number of viable cells was determined at the beginning of experiment, after 1 h (short-term contact) and 24 h (long-term contact). 0.1 cm^3 of sample was plated (spread plate method) directly on a nutrient agar and another 1 cm^3 of sample which were serially diluted according to the standard dilution method (10^{-1}–10^{-6}) was inoculated onto the nutrient agar plates in triplicate [3]. These plates were incubated at 37±0.1º C for 24 h, and then the bacterial colonies were counted. The number of viable cells was reported as CFU cm^{-3}. The antibacterial activity was expressed as reduction of log CFU as compared to control.

The leaching of Ag^+, Cu^{2+} and Zn^{2+} from the zeolite samples was determined after 24 h of contact. The pH value of media after 24 h of contact was measured with Mettler Toledo FE20/FG2. Metal content in all experiments was determined in filtrate by an atomic absorption spectrophotometer (Varian Spectra 55B).

RESULTS AND DISCUSSION

All metal-loaded zeolites contained about 0.25 mmol metal/g zeolite. Their antibacterial activity was tested against *E. coli*. In both control systems (with parent CLI and zeolite A) no significant changes was observed when comparing the number of bacteria at the beginning and end of the experiment showing that zeolite A and CLI itself do not show antibacterial activity.

Antibacterial activity of Zn-A and Zn-CLI applied at different concentration is given in Table 2. For Zn-A only for the concentration of 1 g/100 cm^3 the antibacterial activity was found (0.50 log CFU). Antibacterial activity of Zn-CLI significantly increases with increasing of Zn-CLI amount after 1 h of the contact. Thus, for the concentration of 0.1 g/100 cm^3 the reduction is

2.79 log CFU whereas for 1 g/100 cm^3 it is 5.09 log CFU. After 24 h of contact both Zn-A and Zn-CLI exhibit bactericidal effect for all studied concentrations.

Table 2. Reduction log CFU found for Zn-CLI and Zn-A for 1 h and 24 h compared to the control and final pH values.

Zeolite	Concentration ($g/100\ cm^3$)	Reduction log CFU		pH
		1 h	24 h	
Zn-A	0.1	-0.09	7.03	8.73
	0.5	-0.06	7.03	9.21
	1	0.50	7.03	9.25
Zn-CLI	0.1	2.79	7.59	6.68
	0.5	4.79	7.59	6.62
	1	5.09	7.59	6.10

E. coli: t_0(Zn-A)=$1.68 \cdot 10^7$ CFU cm^{-3}; t_0(Zn-CLI)= $5.00 \cdot 10^6$ CFU cm^{-3}; pH_{start}=7.81

The results of the antibacterial activity of Cu-A and Cu-CLI are presented in Table 3. After 1 h Cu-A shows the antibacterial activity for all examined concentrations. Antibacterial activity of Cu-CLI increases with increasing the used amount of zeolite and it differs from the activity of Cu-A. Thus, for the concentration of 1g/100 cm^3 the reduction of 5.90 and 4.67 log CFU for Cu-A and Cu-CLI were found, respectively. After 24 h of contact the zeolite samples show bactericidal effect, except from Cu-A at the lowest examined amount.

Table 3. Reduction log CFU found for Cu-CLI and Cu-A for 1 h and 24 h compared to the control and final pH values.

Zeolite	Concentration [g zeolite/100 cm^3]	Reduction log CFU		pH
		1 h	24 h	
Cu-A	0.1	3.77	7.43	7.57
	0.5	4.24	7.43	7.38
	1	5.90	7.43	7.30
Cu-CLI	0.1	3.55	5.82	7.41
	0.5	4.59	6.56	7.01
	1	4.67	6.56	6.28

E. coli: t_0(Cu-A)=$2.10 \cdot 10^7$ CFU cm^{-3}; t_0(Cu-CLI)= $7.35 \cdot 10^6$ CFU cm^{-3}; pH_{start}=7.81

Table 4 shows the antibacterial activity of Ag-A and Ag-CLI after 1 h and 24 h. After 1 h Ag-CLI exhibits bactericidal effect for all examined amounts. Ag-A exhibits similar effect as Ag-CLI (except for the concentration of 0.1/100 cm^3).

Table 4. Reduction log CFU found for Ag-CLI and Ag-A for 1 h and 24 h compared to the control and final pH values.

Zeolite	Concentration [g zeolite/100 cm^3]	Reduction log CFU		pH
		1 h	24 h	
Ag-A	0.1	4.87	7.11	7.48
	0.5	7.42	7.11	7.38
	1	7.42	7.11	7.30
Ag-CLI	0.1	7.26	7.59	7.97
	0.5	7.26	7.59	8.62
	1	7.26	7.59	8.71

E. coli: t_0(Ag-A)=$3.37 \cdot 10^7$ CFU cm^{-3}; t_0(Ag-CLI)= $1.29 \cdot 10^7$ CFU cm^{-3}; pH_{start}=7.81

The leached amount of metal was measured by AAS after 24 for all examined systems. It has been found that the leaching is in the range 0.2-3% indicating that metal itself could not be responsible for the antibacterial activity. Table 5 shows the results obtained for the highest values of the used zeolite amounts.

Table 5. Percentage of the leached metal from the zeolite.

Zeolite	[%]		
	Cu^{2+}	Zn^{2+}	Ag^{+}
CLI	1.0	1.4	1.8
Zeolite A	0.5	0.2	0.6

The final pH values in the examined systems differ for different metals and zeolite types. It can be noticed that *E. coli* optimally grows in the pH range 6-8. [4] Since the most of the examined systems have the final pH in this range, the change of pH cannot be regarded as a reason for the decay of bacteria.

CONCLUSION

Cu-, Zn- and Ag-containing natural clinoptilolite and zeolite A show antibacterial activity against *E. coli* DSM 498. The activity depends on metal type and type of zeolites. For 1 h of contact the sensitivity of *E. coli* decreases in the range (Ag-A, Ag-CLI) < Cu-A < Cu-CLI < Zn-CLI < Zn-A. All zeolites show bactericidal effect after 24 h of contact. The results show that the natural clinoptilolite and zeolite A are promising for the preparation of bactericidal agents.

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