Antibacterial activity of heavy metal-loaded natural zeolite

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The antibacterial activity of natural zeolitized tuffs containing 2.60 wt.% Cu2+, 1.47 Zn2+ or 0.52 Ni2+ were tested. Antibacterial activities of the zeolites against Escherichia coli and Staphylococcus aureus were tested after 1 h and 24 h of exposure to 1 g of the zeolite in 100 ml of three different media, namely Luria Bertani, synthetic wastewater and secondary effluent wastewater. The antibacterial activities of the zeolites in Luria Bertani medium were significantly lower than those in the other media and negatively correlated with the chemical oxygen demand of the media. The Ni-loaded zeolite showed high leaching of Ni2+ (3.44–9.13 wt.% of the Ni2+ loaded) and weak antibacterial activity in the effluent water. Since Cu-loaded zeolite did not leach Cu2+ and the leaching of Zn2+ from Zn-loaded zeolite was low (1.07–1.61 wt.% of the Zn2+ loaded), the strong antibacterial activity classified the Cu- and Zn-loaded zeolite as promising antibacterial materials for disinfection of secondary effluent water.

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1. Introduction

The removal of heavy metals from polluted water is of a great importance in order to reduce their hazardous effects on the environment due to their non-biodegradability and tendency to accumulate in living organisms [1]. Sorption has been considered as very suitable method for the removal of heavy metals from wastewater. During the last two decades, the use of natural sorbents is gaining much attention in the sorption of heavy metals from wastewater [2]. Accordingly, the natural zeolitized tuffs are promising materials for the sorption taking into account their availability, low cost, chemical inertness, porosity, large surface area, non-toxicity and environmental acceptability. Zeolites are crystalline hydrated aluminosilicates with structures based on an infinitely extended three-dimensional network consisting of [AlO4]4− and [SiO4]4− connected mutually via all oxygen atoms. The substitution of Al3+ for Si4+ results in negatively charged aluminosilicate lattice, in which inorganic metal cations of mainly Na+, K+, Ca2+ and Mg2+ render electroneutrality [3]. The cations are movable and exchangeable by other metal ions present in the solution.

Previous detailed studies showed that the natural zeolitized tuffs could serve as a sorbent for the removal of copper, zinc and nickel from aqueous media [4–6]. After the sorption of Cu2+, Zn2+ and Ni2+, such heavy metal-loaded natural zeolitized tuffs could find application in the disinfection of wastewater [7,8]. The divalent copper, zinc and nickel ions are essential micronutrients for bacteria, required as trace elements at nano- to micromolar concentrations. However, at millimolar concentrations Cu2+, Zn2+ and Ni2+ are toxic to majority of wild-type bacteria and can be tolerated by minority of bacterial strains [9–12]. Although the Cu2+, Zn2+ and Ni2+ present in the solution are bactericidal, the cations present in the clinoptilolite lattice could exhibit different toxicity than free ions [13]. The aim of this study was to test the antibacterial activity of the natural zeolitized tuff after loaded with Cu2+, Zn2+ or Ni2+ against representative pathogenic Gram-negative bacteria Escherichia coli and Gram-positive bacteria Staphylococcus aureus, having in mind a possible application of the spent sorbent, particularly for the removal of pathogenic bacteria in the tertiary stage of wastewater treatment.

2. Materials and methods

2.1. Preparation and characterization of the zeolites

The natural zeolitized tuff containing 70 wt.% of clinoptilolite (quartz and feldspar were major impurities) was obtained from the sedimentary deposit Zlatokop, Serbia [6]. The particle size of the sample was in the range of 0.063–0.1 mm. The zeolitized tuff was firstly converted into the Na-rich form in order to improve the clinoptilolite cation exchange capacity. The Na-form of natural zeolitized tuff (NaNZ) was then used for preparation of metal-loaded natural zeolitized tuffs. The metal-loaded zeolites were prepared using 1.0 g of the NaNZ and 100 ml of 6 mM MCl2 (M = Cu, Zn or Ni; p.a., Aldrich) aqueous solution. The suspensions were
shaken for 24 h in a thermostated water bath at 30°C, and the metal-loaded zeolites were then recovered by filtration. The metal-loaded zeolites were washed with distilled water until negative reaction to Cl⁻ ions was reached. The elemental analyses of the natural and modified zeolite were performed using energy dispersive X-ray spectroscopy (EDS) by a scanning electron microscope (Jeol, JSM-6610LV). The NaNZ and metal-loaded zeolites were air dried at 105°C for 2 h and sterilized by autoclaving (121°C, 15 min) prior to the antibacterial activity tests.

2.2. Antibacterial activity tests

The antibacterial activity of CuNZ, ZnNZ and NiNZ was tested against representative pathogenic bacteria which can be found in wastewater: Gram-negative bacteria E. coli (strain DSM no. 498) and Gram-positive bacteria S. aureus (strain DSM no. 799), obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

The antibacterial assay was carried out in three different liquid media: Luria Bertani (LB) medium, synthetic wastewater and real effluent water from the secondary stage of the biological wastewater treatment plant. The composition of LB medium was prepared using (in mL⁻¹ of distilled water): tryptone 10,000 (BioLife 4122292); yeast extract 5000 (BioLife 4122220); NaCl 10,000 (Kemika 1417506). Chemical oxygen demand (COD) of the LB medium was measured spectrophotometrically (Hach, DR 2500) using the reactor digestion method (Hach method 8000) in order to estimate the amount of organic compounds in the medium as 14,000 mg L⁻¹.

The composition of synthetic wastewater [5] was (in mL⁻¹ of distilled water): Na-propanol 1000 (Fluka 81992); peptone 100 (BioLife 4122259); MgSO₄ 7H₂O 10 (Kemika 1325006); CaCl₂ 6 (Lach-Ner 30974); KCl 30 (Kemika 1120907); yeast extract 10 (BioLife 412220); KH₂PO₄ 20 (Kemika 1112408); COD 1.206. The real effluent water was obtained after the secondary stage of the biological wastewater treatment at wastewater treatment plant in Zagreb, Croatia. The fresh sample of effluent water was filtered through a Buchner funnel with filter paper (blue band) and Sartorius nitrocellulose filters of pore diameter 0.45 μm within 2 h after the sampling. The chemical composition of the effluent water was (in mL⁻¹): total nitrogen (T-N) 28.3; total phosphorus (T-P) 2.27; COD 31.4. The T-N and T-P were measured spectrophotometrically using the persulfate digestion method (Hach method 10072) and ascorbic acid method with acid persulfate digestion (Hach method 10127), respectively. The pH of all media was adjusted to 7.0 ± 0.2 with 1 M NaOH or 1 M HCl before autoclaving to prevent the possible influence of different initial pH to bacteria and make the results comparable. The pH value of media was measured with the WTW, 330 pH-meter. All media were sterilized by autoclaving (121°C, 15 min) prior to the antibacterial activity tests.

In order to examine the antibacterial activity of metal-loaded zeolites, the bacteria E. coli and S. aureus were pre-grown on LB agar for 16 h at 37.0 ± 0.1°C to obtain the cultures in log phase of growth. The bacterial biomass was then suspended in the sterile 0.05 M NaCl solution. One mL of the suspended biomass of E. coli or S. aureus was inoculated into Schott bottles which contained 100 mL of LB medium, synthetic wastewater or effluent water, giving the initial number of colony forming units (CFU) of 10⁶-10⁷ mL⁻¹. To each of the bottles, 1.0 g of the autoclaved metal-loaded zeolites or metal chloride salts giving the final metal cation concentration in the solution equivalent to the amounts loaded to the zeolites were added. The control bottles were left without addition of the zeolites or metal salts. To check the possibility of antibacterial activity of NaNZ, the experimental bottles containing 1.0 g of the autoclaved NaNZ were set up. The bottles were sealed and incubated for 24 h in a water bath (Memmert, WNB22) at 37.0 ± 0.5°C with shaking at 70 rpm to assure the complete mixing.

The number of viable cells was determined at the beginning of experiment, after short-term exposure of 1 h (corresponding to the lag phase of bacterial growth) and long-term exposure of 24 h (corresponding to the stationary phase of bacterial growth). The Gram staining followed by light microscopy (Olympus, CX21) was performed in order to estimate the range of high or low bacterial numbers in the bottles and the immobilization of bacteria onto the zeolites. The immobilization of bacteria onto zeolites was confirmed by scanning electron microscopy (Jeol, JSM 5300). For the determination of high bacterial numbers, a 1 mL of the suspension was serially diluted (10⁻¹ to 10⁻⁹) in triplicate in sterile 0.05 M NaCl and volumes of 0.1 mL were aseptically inoculated onto the LB agar plates (spread plate method). For the determination of low bacterial numbers, a 10, 20 and 30 mL of the suspension was filtered through 0.20 μm Sartorius sterile nitrocellulose filters and the filters were aseptically placed onto the LB agar. The LB agar plates were incubated at 37.0 ± 0.1°C for 24 h when the growth of colonies was complete. After the incubation period, the bacterial colonies were counted and the number of viable cells was reported as CFU mL⁻¹. In the experiments with NaNZ, the numbers of planktonic and immobilized bacteria onto NaNZ were determined as described previously [5], to determine the number of total cells in the bottles.

To analyse the results, the antibacterial activity of the metal-loaded zeolites or metal chloride salts was expressed as the percent reduction in the number of E. coli and S. aureus after the contact periods of 1 h and 24 h as compared to the corresponding controls. The statistical analyses were carried out using Statistica Software 9.1 (StatSoft, Tulsa, USA). The numbers of viable bacterial cells after incubation in different media were logarithmically transformed beforehand to normalize distribution and to equalize variances of the measured parameters. The comparisons between log CFU were done using the one-way analysis of variance (ANOVA) and subsequently the post-hoc Duncan test was performed for the calculations concerning pair-wise comparisons. The significantly different values were expressed as: A indicates different bacterial numbers in the sample with respect to corresponding control, B indicates different bacterial numbers in the sample with respect to LB medium with metal-loaded zeolites, C indicates different bacterial numbers in the sample with respect to synthetic wastewater with metal-loaded zeolites, D indicates different bacterial numbers in the sample with respect to effluent water with metal-loaded zeolites. The post-hoc Duncan test was also performed for the final pH values of the media. The correlation between COD of media and percentage of bacterial reduction was estimated by Spearman correlation analysis. Statistical decisions were made at a significance level of p < 0.05.

The leaching of Cu²⁺, Zn²⁺ and Ni²⁺ from CuNZ, ZnNZ and NiNZ, respectively was determined after 24 h of contact of the bacteria with the effluent water. The suspension was filtered through 0.20 μm Sartorius syringe filters and the effluent water was analyzed by atomic absorption spectrophotometer (AAS Varian, Spectra AA 55b).

3. Results

3.1. Characterization of the zeolites

Complete chemical compositions of the clinoptilolite phase of natural zeolitized tuff, NaNZ, CuNZ, ZnNZ and NiNZ are given in Table 1. A previous detailed EDS analysis of the metal-loaded natural zeolitized tuffs showed that Cu²⁺, Zn²⁺ and Ni²⁺ replace only the Na⁺ ions in the clinoptilolite phase [14]. The replacement occurs through an ion exchange of Na⁺ in the zeolite by the M²⁺ ions in the solution so that the Na⁺ concentration decreases by the M²⁺...
uptake. The difference in the M$^2$ of the zeolite content showed that the clinoptilolite exhibits different affinity towards the examined metal cations.

### 3.2. Antibacterial tests

In the control bottles without metal-loaded zeolites with different liquid media after 1 h of incubation no significant changes in the bacterial numbers were observed when compared to the numbers at the beginning of the experiments. After 24 h of incubation, the *E. coli* and *S. aureus* showed progressive growth in the controls with LB medium with increase of 2.60 and 3.10 log CFU, respectively. The growth of *E. coli* and *S. aureus* in the controls with synthetic wastewater after 24 h of incubation was lower with increase of 1.94 and 0.80 log CFU, respectively. In the controls with effluent water after 24 h of incubation, a slight decrease in the numbers of *E. coli* (0.02 log CFU) and significant decrease in the numbers of *S. aureus* were observed (1.21 log CFU). The decrease in the numbers of *S. aureus* in the controls with effluent water can be explained by dead proportion of bacteria in the stationary phase of growth in the unfavorable composition of the medium (low COD). *S. aureus* only survives but cannot multiply in wastewater [15] and higher decay rate in freshwater at higher temperatures [16] were reported.

Antibacterial activity of CuNZ and corresponding amount of Cu$^{2+}$ against *E. coli* and *S. aureus* in different liquid media are shown in Table 2. The CuNZ resulted in reduction of the bacterial numbers when compared to the control after 1 h of contact, which was pronounced after 24 h of contact. The reduction in the bacterial numbers increased from LB medium, followed by synthetic wastewater and effluent water. In effluent water after 24 h of contact, the *S. aureus* was more resistant to CuNZ. After 24 h of contact with bacteria with CuNZ, there was no detectable Cu$^{2+}$ leached from CuNZ in the effluent water (0.000 mg 100 mL$^{-1}$). The amount of Cu$^{2+}$ that corresponded to the amount of Cu$^{2+}$ present in CuNZ in the effluent water resulted in higher reduction of both bacteria after 1 h and 24 h of exposure than CuNZ.

The reductions in the number of *E. coli* and *S. aureus* viable cells by ZnNZ and corresponding amount of Zn$^{2+}$ in different liquid media are shown in Table 3. Although low reduction in bacterial numbers by ZnNZ was observed after 1 h of contact, the reduction after 24 h of contact was similar to CuNZ. The reduction in the bacterial numbers increased from LB medium to synthetic wastewater and the effluent water, where the reduction in the numbers of *S. aureus* cells was not significantly different for the synthetic wastewater and the effluent water for 1 h and 24 h contact periods. In the effluent water the *S. aureus* was more resistant to ZnNZ than *E. coli*. The concentration of leached Zn$^{2+}$ from ZnNZ in the effluent water after 24 h of contact was 0.236 mg 100 mL$^{-1}$ (1.61 wt.% of the Zn$^{2+}$ loaded) in the experiment with *E. coli* and 0.158 mg 100 mL$^{-1}$ (1.07 wt.% of the Zn$^{2+}$ loaded) in the experiment with *S. aureus*. The Zn$^{2+}$ in effluent water resulted in higher reduction of both bacteria after 1 h and 24 h of exposure than ZnNZ.

The results of reduction in the bacterial numbers by NiNZ and corresponding amount of Ni$^{2+}$ in different liquid media are shown in Table 4. After 1 h of contact, a negligible reduction in the bacterial numbers by NiNZ was observed in all the examined liquid media. The reduction in the bacterial numbers was higher after 24 h, but did not reach even 20% in any liquid media. The antibacterial activity of NiNZ was also lower in LB medium when compared to synthetic wastewater and effluent water. The concentration of leached Ni$^{2+}$ from NiNZ in the effluent water after 24 h was moderate in experiment with *E. coli* (0.179 mg 100 mL$^{-1}$ corresponding to 3.44 wt.% of the Ni$^{2+}$ loaded) and very high in experiment with *S. aureus* (0.475 mg 100 mL$^{-1}$ corresponding to 9.13 wt.% of the Ni$^{2+}$ loaded). The Ni$^{2+}$ in effluent water showed better antibacterial activity than NiNZ, but did not exceed 95% even after 24 h of exposure, in the effluent water the *S. aureus* was more resistant to NiNZ and Ni$^{2+}$ than *E. coli*.

The difference in final pH among control and experimental bottles was not higher than 0.19, 1.52, 1.59 and 0.79 pH units for LB medium, synthetic wastewater, effluent with metal-loaded zeolites and effluent with metal ions, respectively. Therefore, the changes in pH values were not the reason for the reduction in the bacterial numbers. In the experiments with NaNZ (data not shown) after 24 h of contact in effluent water the majority of *E. coli* (1.83 ± 0.14 × 10$^7$ CFU g$^{-1}$ of dry NaNZ) and *S. aureus* (2.36 ± 0.32 × 10$^7$ CFU g$^{-1}$ of dry NaNZ) was immobilized onto NaNZ (Fig. 1a and b), while the minority of bacteria rest as planktonic bacteria in the supernatant. In the experiments with metal-loaded zeolites no immobilization or only sporadically few cells showed evidence of immobilization.
immobilized bacteria were found (data not shown) and the bacterial population was represented by planktonic cells. The total number of bacteria in bottles with NaNZ was not significantly different than in the corresponding controls, showing that NaNZ had no antibacterial activity itself.

4. Discussion

The percent reduction in the number of bacterial cells in the presence of CuNZ, ZnNZ and NiNZ showed significantly negative correlation with the COD of liquid media used in assay ($R = -0.993$, $-0.987$ and $-0.813$, respectively). The results of antibacterial activity of metal-loaded zeolites in the effluent water were much closer to the results obtained in the synthetic wastewater than to the results in the LB medium. Therefore, the results of antibacterial assay performed in the nutrient rich media used for cultivation of bacteria should be taken with reserve if results are aimed to be applied in a nutrient depleted media. The antibacterial activity of silver-loaded zeolite was inhibited by the addition of amino acids and yeast extract [17]. Cations of heavy metals can form insoluble compounds with chloride (originating from tryptone and yeast extract) anions. These could explain lower antibacterial activity of metal-loaded zeolites in LB medium when compared to the other media.

The significantly higher reduction of bacteria by metal ions than by metal-loaded zeolites is explained with the higher antibacterial activity of the free ions in the solutions as compared to the metal ions present in the clinoptilolite lattice. Chlorine ions from the metal chloride salts may have a synergistic antibacterial effect. The toxicity of silver ions against E. coli was increased in the presence of 20–30 g L$^{-1}$ of NaCl [18]. The concentrations of chlorine ions in present study were 48–193 times lower. Moreover, there was no significant difference in antibacterial activity of the metal sulphate salts as compared to the metal chloride salts (data not shown). Therefore, the contribution of chlorine ions to the antibacterial activity of metal ions could be safely excluded.

The concentration of leached metals in the effluent water after 24 h of contact decreased in the order Ni$^{2+} > $Zn$^{2+} > $Cu$^{2+}$. The intensity of leaching can be explained by the selectivity sequence of the investigated natural zeolitized tuff towards metal cations, which was Cu$^{2+} > $Zn$^{2+} > $Ni$^{2+}$ [4–6]. In abiotic experiment with 0.05 M NaCl of pH 7.0 at 37 $^{\circ}$C during 24 h the concentration of leached Zn$^{2+}$ from the same sample of ZnNZ was 0.067 mg 100 mL$^{-1}$ [5]. In the present study, the concentration of leached Zn$^{2+}$ from ZnNZ was 3–7 times higher. This difference could be explained either by the role of bacterial cells in the amount of Zn$^{2+}$ leached or by the influence of metal ions present in different liquid media. The concentration of leached silver ions from silver-containing zeolites was also higher in the

### Table 3

Percent reduction in the number of E. coli and S. aureus cells after contact with ZnNZ or Zn for 1 h or 24 h in different media as compared to the controls.

<table>
<thead>
<tr>
<th>Medium</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduction 1 h (%)</td>
<td>Reduction 24 h (%)</td>
</tr>
<tr>
<td>LB medium + ZnNZ</td>
<td>4.90 ± 0.60$^A$</td>
<td>5.05 ± 0.64$^A$</td>
</tr>
<tr>
<td>Synthetic wastewater + ZnNZ</td>
<td>6.27 ± 1.22$^A$</td>
<td>93.92 ± 0.55$^A$</td>
</tr>
<tr>
<td>Effluent + ZnNZ</td>
<td>8.09 ± 1.56$^{A,B,C}$</td>
<td>95.07 ± 0.12$^{A,C}$</td>
</tr>
<tr>
<td>Effluent + NaNZ</td>
<td>28.93 ± 1.48$^{A,B,C,D}$</td>
<td>100.00 ± 0.00$^{A,B,C,D}$</td>
</tr>
</tbody>
</table>

$t_0$ E. coli (10$^6$ CFU mL$^{-1}$) = 3.49 ± 1.02; $t_0$ S. aureus (10$^6$ CFU mL$^{-1}$) = 1.51 ± 0.26; significantly different as compared to $^A$ – corresponding control, $^B$ – LB medium + ZnNZ, $^C$ – synthetic wastewater + ZnNZ, $^D$ – effluent + ZnNZ.

### Table 4

Percent reduction in the number of E. coli and S. aureus cells after contact with NiNZ or Ni for 1 h or 24 h in different media as compared to the controls.

<table>
<thead>
<tr>
<th>Medium</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduction 1 h (%)</td>
<td>Reduction 24 h (%)</td>
</tr>
<tr>
<td>LB medium + NiNZ</td>
<td>1.39 ± 0.31$^A$</td>
<td>3.20 ± 0.27$^A$</td>
</tr>
<tr>
<td>Synthetic wastewater + NiNZ</td>
<td>1.38 ± 0.35</td>
<td>19.27 ± 0.55$^A$</td>
</tr>
<tr>
<td>Effluent + NiNZ</td>
<td>1.74 ± 0.41$^A$</td>
<td>18.48 ± 0.62$^A$</td>
</tr>
<tr>
<td>Effluent + NaNZ</td>
<td>12.25 ± 0.61$^{A,B,C,D}$</td>
<td>94.18 ± 1.84$^{A,B,C,D}$</td>
</tr>
</tbody>
</table>

$t_0$ E. coli (10$^6$ CFU mL$^{-1}$) = 3.49 ± 1.02; $t_0$ S. aureus (10$^6$ CFU mL$^{-1}$) = 1.51 ± 0.26; significantly different as compared to $^A$ – corresponding control, $^B$ – LB medium + NiNZ, $^C$ – synthetic wastewater + NiNZ, $^D$ – effluent + NiNZ.

**Fig. 1.** Immobilized cells of E. coli (a) and S. aureus (b) onto NaNZ.
presence of *E. coli* than in abiotic control. The phenomenon was explained by the contact of bacterial cells with silver-containing zeolites and active uptake of silver ions by the bacteria, which consequently caused the damage of cells [17,19].

The silver ions leached from the silver-loaded zeolite X were reported as responsible for the antimicrobial action against *E. coli* and *S. aureus* in tryptone soya broth [19]. In the present study the best antibacterial activity in the effluent water was observed with CuNZ for which no leaching of the Cu²⁺ was detected. Moreover, the amount of metal leaching was reversal to the antibacterial activity. This suggested that the leaching of metal ions in the effluent water was not responsible for the antibacterial activity. It is likely that the particles of metal-loaded zeolites act bactericidal itself, without the need that metals are leached from material to the liquid medium. It can be presumed that bacteria come into contact with metal-loaded zeolites and active take up metal ions, which consequently damage the cells.

The stability and slow release of desired metal ions from bactericidal particles are main characteristics which give them the advantage in use when compared to the free metal ions. Due to high leaching of Ni²⁺ (3.44–9.13 wt.% of the Ni²⁺ loaded) from NiNZ and low antibacterial activity of particles, the NiNZ cannot be considered as a suitable material for the use in effluent water. As 0.00 wt.% of the Cu²⁺ loaded and less than 1.61 wt.% of the Zn²⁺ loaded in the zeolite were released from the materials in effluent water during the 24 h exposure, there were sufficient concentrations of Cu²⁺ and Zn²⁺ available for the subsequent exposures. Therefore, the CuNZ and ZnNZ can be regarded as promising antibacterial materials for disinfection of secondary effluent water.

In the examined secondary effluent water 1.3 × 10⁴ CFU mL⁻¹ of heterotrophic bacteria, 7.0 × 10³ ml⁻¹ of total coliform bacteria, 5.9 × 10² ml⁻¹ of faecal coliform bacteria and 8 × 10¹ ml⁻¹ of faecal streptococci were measured. In the present study the numbers of *E. coli* and *S. aureus* in effluent water after 24 h of exposure to CuNZ and ZnNZ were reduced for six orders of magnitude. Since good antibacterial activity of CuNZ and ZnNZ was observed against Gram-negative and Gram-positive bacteria, it can be supposed that the safe disinfection of secondary effluent can be achieved by the use of these materials.

5. Conclusions

The antibacterial activity of metal-loaded natural zeolitized tuffs against *E. coli* and *S. aureus* was dependent on the following factors in decreasing order: type and COD of the liquid medium, type of material and species of bacteria. Due to high leaching of Ni²⁺ from NiNZ and low antibacterial activity of NiNZ, the NiNZ cannot be suggested as a suitable material for the use in disinfection of effluent water. The CuNZ and ZnNZ showed no or low leaching of Cu²⁺ and Zn²⁺, respectively and reduced the bacterial numbers for six orders of magnitude in effluent water. The CuNZ and ZnNZ could find a novel application in the removal of pathogenic bacteria in the tertiary stage of wastewater treatment.

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