



Full length Article

Natural sorbents modified by divalent Cu^{2+} - and Zn^{2+} - ions and their corresponding antimicrobial activity

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ABSTRACT

The objective of this study was to investigate the modification of materials used in wastewater treatment for possible antimicrobial application(s). Granulated activated carbon (GAC) and natural clinoptilolite (CLI) were activated using Cu^{2+} - and Zn^{2+} - ions and the disinfection ability of the resulting materials was tested. Studies of the sorption and desorption kinetics were performed in order to determine and clarify the antimicrobial activity of the metal-activated sorbents. The exact sorption capacities of the selected sorbents, GAC and CLI, activated through use of Cu^{2+} - ions, were 15.90 and 3.60 mg/g, respectively, while for the materials activated by Zn^{2+} - ions, the corresponding capacities were 14.00 and 4.72 mg/g. The desorption rates were 2 and 3 orders of magnitude lower than their sorption efficacy for the Cu^{2+} - and Zn^{2+} -activated sorbents, respectively. The intermediate sorption capacity and low desorption rate indicated that the overall antimicrobial activity of the metal-modified sorbents was a result of metal ions immobilized onto surface sites. The effect of antimicrobial activity of free ions desorbed from the metal-activated surface may thus be disregarded. The antimicrobial activities of Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI were also tested against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. After 15 min exposure, the highest levels of cell inactivation were obtained through the Cu/CLI and the Cu/GAC against *E. coli*, 100.0 and 98.24%, respectively. However, for *S. aureus* and yeast cell inactivation, all Cu^{2+} - and Zn^{2+} -activated sorbents proved to be unsatisfactory. A characterization of the sorbents was performed by X-ray diffraction (XRD), X-ray photo electron spectroscopy (XPS), and field emission scanning electron microscopy (FE-SEM). A concentration of the adsorbed and released ions was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and mass spectrometry (ICP-MS). The results showed that the antimicrobial performance of the activated sorbents depended on the surface characteristics of the material, which itself designates the distribution and the bioavailability of the activating agent.

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Introduction

The challenge of water purification technologies to meet the growing resistance and variety of pathogenic microorganisms has increasingly become an area of concern within the last two decades. Among the various disinfection processes, numerous biotechnological solutions rely on sorption processes [1,2]. Materials impregnated with different metal ions are known to

be effective antimicrobial carriers [3–6]. The use of inorganic materials carries with it several distinct advantages over organic agents, such as chemical stability, thermal resistance, null toxicity for the user, as well as long and durable action periods [7]. Natural materials, such as aluminosilicates and clays, have been widely tested for microbial cell inactivation due to their superior characteristics (remarkable ion exchange capacity, negative surface charge, diversity in pore size, shape, topology, chemical inertness, and low toxicity) [8]. Montmorillonite adsorbed with copper and zinc ions has been found to be a suitable carrier for antibacterial and antifungal purposes [4,9]. Moreover, clinoptilolite used as a sorbent for the removal of Zn^{2+} - ions has been further

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tested for its antimicrobial activity as a Zn²⁺-activated sorbent [3]. Ahammed et al. reported the antibacterial effect of manganese oxide- and iron hydroxide-coated sand as a dual filter media for both the simultaneous removal of heavy metals and total coliform cells, showing a 99 and 96% removal, respectively [10]. In addition, Pal et al. modified Al₂O₃ and Zn(OH)₂ activated carbon for enhanced *Escherichia coli* removal [11]. Hydroxyapatite adsorbed with silver and copper ions has also been tested as a possible bone replacement and dental implant material [12].

This study focused on the suitability and application of Cu²⁺- and Zn²⁺- activated sorbents against microbial growth in aqueous systems, as recognized for biotechnological operations [3,6,9,10]. In preliminary tests, Cu²⁺- and Zn²⁺- ions were loaded into 11 different materials: 5 natural-vegin (clinoptilolite, sepiolite, bentonite, calcite and quartz); 3 natural-modified (powder activated carbon, granulated activated carbon and activated alumina); and 3 synthetic (artificial zeolite, titanium dioxide and ion exchange resin). The varying antimicrobial aspects of the pairs of sorbents, clinoptilolite (CLI) and granulated activated carbon (GAC), and chemically active agents (Cu²⁺- and Zn²⁺- ions) were then observed [9,12–24]. The antimicrobial activity of Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI sorbents was tested against the Gram-negative bacterium, *Escherichia coli*, the Gram-positive *Staphylococcus aureus*, and the yeast, *Candida albicans*. The aim has been to evaluate Cu- and Zn-activated sorbents to find those most suitable for bacteria and yeast cell removal as well as to define their antimicrobial activity related to their activated surface sites.

The antimicrobial activity of the metal-activated sorbents was observed through the reaction of metal-ions loaded onto the surface of the sorbents and the free metal ions detached from the activated surface in the aqueous phase. A slow and continuous release of metal ions in the microbial environment is critical to ensuring antimicrobial efficacy [25]. Metal activated sorbents tend simultaneously to attract the negatively charged membranes of bacteria to the surface of the material, where the positively charged metal ions inactivate microbial cells or disable them from replicating [3,26]. Since the desorption rate was shown to be low for the Cu²⁺- and Zn²⁺- activated sorbents, overall antimicrobial activity was found to be related to the effect of metal-ion immobilization on the sorbent surface.

In a recent study, silver activated materials were tested for antimicrobial activity and both the influences of Ag⁺- ions were observed, namely cell reduction as a result of the free ions released into the solution and the antimicrobial effect of the ions on the surface of the sorbent [13]. Rather than being mutually exclusive, these two steps are complementary. Malachova et al. tested montmorillonite as an activated carrier and found that it gradually released active substances, such as Ag⁺-, Cu²⁺-and Zn²⁺- ions, leading to antimicrobial activity [4]. Hrenovic et al. also have pointed out that metal-loaded zeolite itself acts a bactericide [3]. The literature thus far has highlighted the importance of the role of the solid phase in influencing the surface characteristics of the material [5,7,14], as it directly determines the distribution and the accessibility of the activating agent [15]. For this reason, only antimicrobial tests on the solid phase (metal-activated sorbents) have been performed here due to the correspondingly low desorption rate of the metal-ions from the selected materials. The main objectives of the present work were the following:

- i) carrying out and testing modification of the sorbents through the simple metal loading process (Cu²⁺- and Zn²⁺-activation process);
- ii) creating a selection of the most suitable sorbents (as from adsorption and desorption kinetic studies);
- iii) investigating structural changes of the Cu²⁺- and Zn²⁺-treated sorbents; and

- iv) performing antimicrobial tests of the Cu²⁺- and Zn²⁺-activated materials.

Material and methods

Material selection and preparation, including physico-chemical properties of the materials, have been provided in our previous study [13]. The characterization of the native and modified sorbents was performed by X-ray diffraction (XRD) and X-ray photo electron spectroscopy (XPS), under the same operating conditions [13]. The analytical procedure of activation (adsorption) and desorption processes, as well as the calculation of sorption and desorption capacity, were described previously [13]. The concentration of adsorbed and released ions was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and mass spectrometry (ICP-MS). Microbiological tests and the estimation of antimicrobial activity were evaluated against *E. coli*, *S. aureus* and *C. albicans*, using a standard dilution method [13].

Results and discussion

Preliminary investigations

The sorption and desorption capacities of selected sorbents loaded with Cu²⁺- or Zn²⁺- ions after 24 h are presented in Table 1. In total, 22 metal-sorbent combinations were obtained in the metal-ion activation process. The efficacy of the activation process was expressed as based on the sorption capacity (SC).

The range of the SC values of the tested materials were 1.70–15.9 mg/g for Cu²⁺- ions and 2.46–14.0 mg/g for Zn²⁺- ions. When comparing the SC and DC values of a given material against a metal ion, the same affinity trend was observed, i.e., Zn²⁺ > Cu²⁺, which agrees with the results of other such studies [4]. The affinity of all the tested materials for a given metal ion was also analyzed and the results shown in the lower section of Table 1. The desorption studies provide information on the concentration of ions released into the solution. The range of the DC values was 0.004–0.553 mg/g for Cu²⁺- and 0.003–1.04 mg/g for Zn²⁺- ions. These results indicate that the DC values for Cu²⁺- ions were nearly 3 (for activated alumina) to 2000 (for ion exchange resin), times lower than the SC values of the same material, whereas, for Zn²⁺- ions, they were found to be from 14 (for bentonite) to 4000 (for ion exchange resin) times lower.

Selection of the appropriate sorbent and activating agent is subject to several parameters: SC value (the quantity of the surface-loaded activating agent) [16–18]; desorption kinetics (the stability of the sorbent – the slow release of free metal ions) [4,19]; chemical state of the activating agent (Cu vs. Cu⁺- and Cu²⁺- ions) [20]; contact time between the microorganisms and the activated sorbent [18,21,22]; structural properties of the material (higher porosity enables more equal distribution of the metal ions throughout the material and more immediate accessibility of the activating agent to the microbial solution) [5,7,14]; particle size (distribution) of the activating agent [23]; and solubility of the activating agent and stability of the sorbent [24].

A successful activation process is the primary step in the preparation and modification of metal-activated sorbents. Radovanovic et al. have demonstrated that a high sorption capacity is not crucial for an effective antimicrobial activity [12]. It has also been pointed up that materials loaded with higher metal contents do not necessarily express significantly higher antimicrobial activity [7,16]. Zhao et al. have also confirmed that a relatively low amount of silver (0.6 wt.%) or copper (0.57 wt.%) ions on minerals act as strong antibacterial agents [19]. Moreover, a slow and controlled release of free metal ions is determined by the

Table 1
Activation (Sorption) and Desorption Capacities of 11 Different Sorbents for Cu²⁺- and Zn²⁺-ions after 24 h of Treatment.

Sorbent			Cu ²⁺		Zn ²⁺	
			SC, mg/g	DC, mg/g	SC, mg/g	DC, mg/g
NATURAL MATERIALS	A. VIRGIN	Clinoptilolite (CLI)	3.60	0.006	4.72	0.013
		Bentonite (B)	1.70	0.125	2.86	0.198
		Sepiolite (S)	4.51	0.005	6.90	0.007
		Calcite (C)	3.76	0.012	4.26	0.025
		Quartz (Q)	1.95	0.36	5.29	0.87
	B. MODIFIED	Powder Activated Carbon (PAC)	4.80	0.02	12.8	0.04
		Granulated Activated Carbon (GAC)	15.9	0.06	14.0	0.20
		Activated Alumina (AL)	1.56	0.553	2.46	0.475
		SYNTHETIC MATERIALS				
		Artificial Zeolite (AZ)	5.54	0.006	9.25	0.005
Titanium dioxide (T)	5.88		0.090	8.85	1.04	
Ion Exchange Resin (IR)	7.37		0.004	12.52	0.003	
Metal ion		SC, mg/g	DC, mg/g			
Cu ²⁺		GAC > IR > T > AZ > PAC > S > CLI > C > Q > B > AL		AL > Q > B > T > GAC > PAC > C > CLI ≈ AZ > S > IR		
Zn ²⁺		GAC > PAC > IR > AZ > T > S > CLI > Q > C > B > AL		T > Q > AL > GAC > B > CLI > PAC > C > S > AZ > IR		

desorption process; the overall antimicrobial activity is directly affected by both processes – sorption and desorption [13,27]. It has been shown that no significant difference in desorption rate exists for materials of lower and higher contents of loaded metal ions [12]. The sorption capacities of hydr(oxide) layered sorbents have been found to be lower than those of metal-activated materials [6].

The chemical state of the activating agent is one of the key determining factors for the removal of microbial cells [7,14,20]. Metallic copper nanoparticles have been shown to be efficient antimicrobial agents in the removal of *E. coli* and *S. aureus* by Cu-modified sepiolite [28]. The toxicity of ionic copper is largely determined by its tendency to alternate between its cuprous (Cu(I)) and cupric (Cu(II)) oxidation states [20]. Moreover, the presence of both copper ions species, Cu⁺- and Cu²⁺- ions released from the metal copper surface leads to higher antimicrobial activity [20]. It has also been discovered that increasing the contact time of microbial solutions with activated sorbents does not always increase antibacterial activity [20].

The effect of structural properties and surface morphology are key factors for the toxicity of metal particles or metal-activated sorbents [3,5,9,29]. The antimicrobial activity of an activated material may differ substantially according to their particle size and distribution [23]. Small particles intensify contacts with microbial cells, making nanoparticles more efficient antimicrobial agents [30]. Gajjar has investigated the effect of particle size of ZnO nanoparticles and concluded that aggregation into larger particles may reduce their antimicrobial activity [23]. Both particle size and shape have been established as being controllable through the addition of various surfactants [31].

One criterion for the selection of an appropriate sorbent (in addition to proven antimicrobial efficacy) is the stability of the sorbent and its lifetime [11]. Apart from metal-ion-activation treatment [2–6], impregnation of materials by oxide or hydroxide films [6,10,11,22], is often used for sorbent modification. The solubility of metal hydr(oxide) is a key issue in the toxicity of metal containing (nano)particles [24]. Such a metal hydr(oxide) coating approach provides more efficient sorbent stability in water due to its low solubility constant [32]. Therefore, the slower release of metal ions from the oxide-activated surface into the solution may affect long-term bacteria removal and allows for extended use of the sorbent [11]. The antimicrobial activities of metal-activated sorbents have been found to be more efficient than metal oxide impregnated sorbents [2,9].

The appropriate selection of sorbents and their microbiological testing is dependent on several factors. In the present study,

attention has been explicitly given to a choice of natural sorbents. Regarding the SC and DC values obtained for Cu²⁺- and Zn²⁺- ions, natural CLI (particle size: 0.4–0.8 mm) and GAC (particle size: 2–4 mm) were selected for further antimicrobial testing. Sorbents of low desorption rates were intentionally selected because the effect of the released Cu²⁺- and Zn²⁺- ions can be ignored in the overall antimicrobial activity of the activated sorbents.

The activation medium itself is one of the key factors in achieving a successful activation process [10,11]. In the literature, it has been repeatedly found that the activation process of the sorption materials is more effective for salts than for the oxides of the same metal [6,22]. The values of the sorption capacity for Cu²⁺- and Zn²⁺- ions in this investigation, when using a GAC activated sorbent, have been consistent with the values obtained in previous investigations while those values obtained for CLI have been significantly lower (as shown in Table 2). The amount of modified materials applied in the microbial assays in this study have been in accordance with the dosages of antimicrobials (10 mg/mL) applied in previous studies [3,5,22]. Contact time can vary from 1 to 24 h [3–5,19,21,22]; however, in our study, it is reduced to 15 min as to minimize the impact of the desorption process. As regards the selection of *E. coli* and *S. aureus* as the tested microbials, they are the most frequently tested bacteria in a biological waste water treatment due to their common risk factor found in natural occurring environments [5,6,21,22,26].

A selection of different materials, the activating agent (Cu²⁺- and Zn²⁺- ions), the chemical components, the sorption capacities of the modified sorbents, and the minimum inhibitory concentration (MIC) used in several previous antimicrobial investigations are presented in Table 2.

Material activation and sorption process efficiency for Cu²⁺ and Zn²⁺ ions

The activation process of Cu²⁺- and Zn²⁺- ions as chemical agents and GAC and CLI as selected sorbents was carried out in singular batch mode. The change in the sorption capacity of Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI, over a 24 h period for the activation process is shown in Fig. 1. The adsorption studies were conducted under acidic conditions (pH = 5.5), ensuring that it was restricted to divalent heavy metal ions [33,34]. The final pH values, after Cu²⁺ and Zn²⁺- ion adsorption, using a GAC sorbent, were 6.50 and 6.70, respectively, while the final pH values for the selected ions, using a CLI sorbent, were 6.80 and 7.10, respectively. Increasing the initial pH (above 5.5) resulted in no significant

Table 2

The Sorption Capacity of Activating Agents in Different Modified Sorbents and Their Antimicrobial Activity (MIC Values).

Material	Activating agent	Chemical component	Sorption capacity (mg/g)	MIC (mg/mL)	Exposure period, h	Pathogen	Ref.
GAC	Cu ⁺	Cu(OH) ₂	16.0	/	16*	<i>E. coli</i>	[6]
GAC	Zn ²⁺	Zn(OH) ₂	3.7	/	16*	<i>E. coli</i>	[6]
GAC	Cu ⁺ , Cu ²⁺	Cu ₂ O, CuO	/	0.5	8	<i>E. coli</i>	[21]
GAC	Cu ²⁺	CuSO ₄ ·5H ₂ O	15.9	/	15**	<i>E. coli</i> , <i>S. aureus</i> , <i>C. albicans</i>	present study
GAC	Zn ²⁺	ZnSO ₄ ·7H ₂ O	14.0	/	15**	<i>E. coli</i> , <i>S. aureus</i> , <i>C. albicans</i>	present study
Clinoptilolite	Cu ²⁺	CuCl ₂	26.0	10.0;10.0	24;24	<i>E. coli</i> ; <i>S. aureus</i> ;	[5]
Clinoptilolite	Cu ⁺	Cu ₂ O	26.0	10.0;10.0	1;1	<i>E. coli</i> ; <i>S. aureus</i> ;	[22]
Clinoptilolite	Cu ⁺	CuSO ₄ ·5H ₂ O	3.60	/	15**	<i>E. coli</i> , <i>S. aureus</i> , <i>C. albicans</i>	present study
Clinoptilolite	Zn ²⁺	ZnO	14.7	10.0;10.0	1;1	<i>E. coli</i> ; <i>S. aureus</i> ;	[22]
Clinoptilolite	Zn ²⁺	ZnCl ₂	13.0	10.0	24	<i>A. junii</i>	[3]
Clinoptilolite	Zn ²⁺	ZnSO ₄ ·7H ₂ O	4.72	/	15**	<i>E. coli</i> , <i>S. aureus</i> , <i>C. albicans</i>	present study
Sepiolite	Cu ²⁺	Cu(NO ₃) ₂ ·6H ₂ O	12.0	/	24	<i>E. coli</i>	[26]
Palygorskite mineral	Cu ²⁺	Cu(NO ₃) ₂	5.7	0.1;0.1	12;12	<i>E. coli</i>	[19]
Montmorillonite	Zn ²⁺	ZnSO ₄ ·7H ₂ O	/	0.55	24	<i>S. aureus</i>	[4]
Montmorillonite	Cu ²⁺	CuSO ₄	34	/	24	<i>E. coli</i>	[25]
Montmorillonite	Cu ²⁺	CuO	/	0.1 × 10 ⁻⁶	24	<i>E. coli</i>	[9]

*Expressed in weeks.

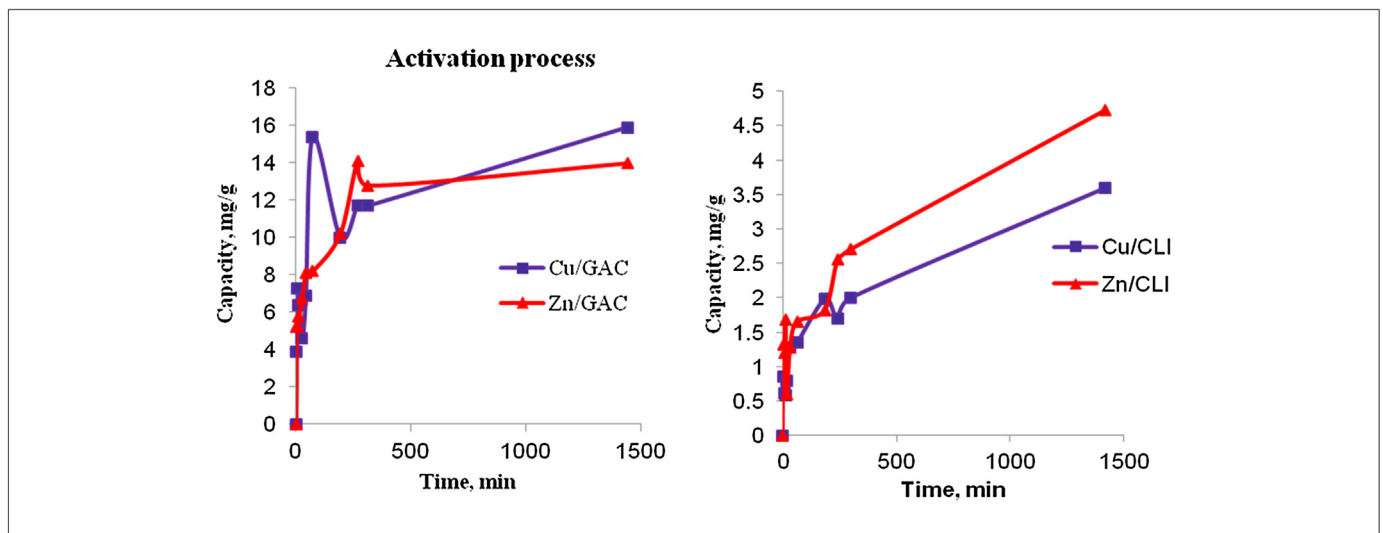
**Expressed in minutes.

change to the sorption capacity. This concurs with findings in previous investigations for the adsorption of Cu²⁺-, Zn²⁺- and Pb²⁺- ions from an aquatic environment [35].

A contact time of 24 h for the adsorption processes was chosen in accordance with the results of previous investigations [33–35]. Although the thermodynamic equilibrium was not met during the chosen contact time (as shown in Fig. 1), the activated sorbents were tested for the state of the activation process achieved. The aim of the activation process was not to obtain maximum metal loadings since it has been found that a high sorption capacity is not crucial for effective antimicrobial activity [12]. The focus of this

investigation has been to clarify the mechanism of antimicrobial activity of surface activated sorbents as modified by metal ions. Therefore, the influence of temperature on Cu²⁺ – and Zn²⁺ –adsorption efficiency has not been taken into consideration. The research at hand has concentrated on clarifying the structural changes and chemical state of the activating agent on surface sites.

The values obtained for the sorption capacities of GAC and CLI sorbents for Cu²⁺- and Zn²⁺- ions were 13.62 and 3.60 mg/g, respectively, and 19.55 and 4.72 mg/g, respectively. The applied activation process for both cations was efficient for the GAC and satisfactory for the CLI sorbent. After 24 h of activation, 71% of the

**Fig. 1.** Activation Process of Gac and Cli Sorbents by Cu²⁺ and Zn²⁺ ions.

initial Cu^{2+} ions were loaded onto the GAC and 65% were adsorbed onto the CLI sorbent. The affinities of the GAC and CLI for Zn^{2+} ions were significantly lower, 39 and 52%, respectively. The change in the sorption capacity as shown over the test time fits pseudo-second order kinetic models. Using the GAC and CLI sorbent, 50% of the initial Cu^{2+} ions had been removed from the solution after 4 and 7 h, respectively, while the removal of Zn^{2+} ions was found to be less efficient.

Desorption of the Cu^{2+} - and Zn^{2+} - ions from the activated surface

Apart from sorption capacities, the desorption of Cu^{2+} - and Zn^{2+} ions is vital in order to confirm that these ions are suitable as chemical activating agents. The desorption kinetic studies focused on changes in the concentration of released Cu^{2+} - and Zn^{2+} - ions over the given test period. The slow release of metal ions confers the stability and longevity upon an activated sorbent. The release of free Cu^{2+} - and Zn^{2+} - ions was simulated in deionized water, at pH 5.5 over a test period of 24 h. The final pH values after the Cu^{2+} and Zn^{2+} - ion desorption, utilizing the GAC sorbent, were 6.20 and 6.40, respectively, while the final pH values for selected ions, utilizing the CLI sorbent, were 6.30 and 6.50, respectively. The results of the studies of the desorption kinetics of Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI are shown in Fig. 2.

The desorption capacities of Cu/GAC and Zn/GAC were 0.06 and 0.20 mg/g, respectively. Cu^{2+} - and Zn^{2+} -activated CLI was found to have lower desorption rates than for metal ions-activated by GAC, i. e., 0.006 and 0.013 mg/g, respectively. Thus, the tendency to release Cu^{2+} - and Zn^{2+} - ions was substantially comparable to that for the selected materials ($\text{Zn}^{2+} > \text{Cu}^{2+}$). Cu/CLI exhibited the lowest desorption rate, while Zn/GAC exhibited the highest desorption rate of all the activated materials. The influence of contact time on the desorption of Cu^{2+} - and Zn^{2+} - ions was evaluated by a kinetic study over 24 h, as presented in Fig. 2. Analyzing the obtained data, the desorption rate of Cu^{2+} - and Zn^{2+} - ions from the activated surface was concluded to be low. Contact time between the metal-activated sorbent and microbial solution was limited to 15 min in order to provide that the metal ions were desorbed in a minimal concentration. For this reason, the equal contact time of desorption process for the microbial tests was tested. In this study, low desorption rates of selected materials were most expedient for the contribution of the activated sites on the surface of the material to the antimicrobial activity.

Structural and surface properties of the activated sorbents

As has been previously noted, one of the main criteria for the selection of antimicrobial materials is the chemical state of the activating agent. Structural and surface analyses of native and metal-activated sorbents provide confirmation of the presence of Cu^{2+} - and Zn^{2+} - ions on the activated surface.

XRD analysis

The results of the XRD analysis of the native, GAC, and activated Cu/GAC and Zn/GAC sorbents are shown in Figs. 3 and 4. Broad peaks centered at 2θ angles of 24.00 and 43.15° evidence the amorphous structure of the GAC sample [21,26]. The analysis shows that copper- and zinc-activated sorbents were subjected to structural changes. The presence of elemental copper (Cu^0) in Cu/GAC was detected by the characteristic peaks at $2\theta = 43.20$ and 50.40° (Fig. 3) [28]. Copper ions (Cu^{2+}) from the activation medium, ($\text{Cu}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ solution) were adsorbed onto the GAC surface and reduced by carbon (a strong reducing agent) to elemental copper (Cu^0) [36]. This reaction explains the chemical state of the activating agent that was loaded and incorporated as metallic copper into the GAC. The diffraction pattern of the Cu/GAC showed peaks at $2\theta = 24.00$ and 43.15°, which were wider than those that appeared for the GAC proper. This is a result of the presence of differing copper oxidation states, elemental copper (Cu^0), and ionic copper (Cu^{2+}) [14]. In addition, this may suggest that the copper was highly dispersed on the GAC but lacked formation of large particles [37]. Copper in the ionic state Cu^{2+} was also registered by the XRD analysis in low intensities (due to its low sorption capacity), its characteristic peaks being at $2\theta = 35.40$ and 38.70° [21]. However, the complementary XPS technique confirmed its presence on the Cu/GAC activated surface. The diffraction pattern of the Zn^{2+} -activated sorbent showed the presence of Zn^{2+} ions in the chemical form of ZnO and zinc-sulfate-hydroxide-hydrate ($\text{Zn}_4\text{SO}_4(\text{OH})_6 \cdot 0.5\text{H}_2\text{O} / \text{ZnSO}_4 \cdot 3\text{Zn}(\text{OH})_2 \cdot 0.5\text{H}_2\text{O}$) (Fig. 4).

The XRD patterns of native, CLI, and activated, Cu/CLI and Zn/CLI, sorbents are shown in Figs. 5 and 6. The patterns display no significant structural changes between the natural and activated sorbents. Moreover, the low intensities of the characteristic peaks for copper ($2\theta = 35.55$ and 38.90°) in Fig. 5 indicate lower amounts of metal ions in the clinoptilolite framework and a higher dispersion of the metal inside the zeolite cavities and channels

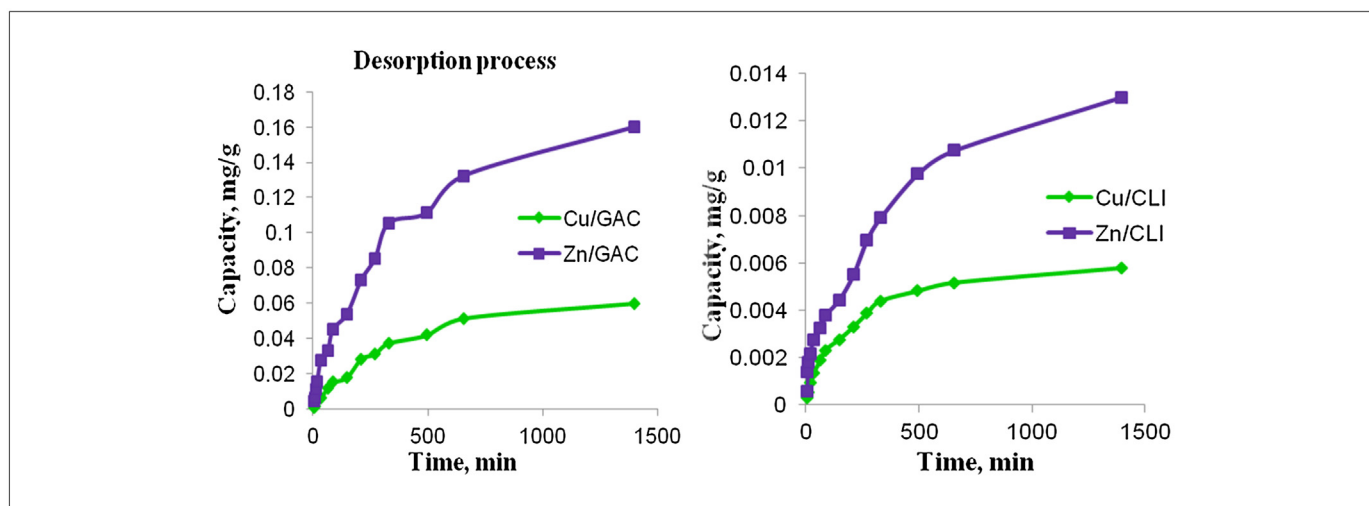


Fig. 2. Desorption of Cu^{2+} and Zn^{2+} ions from the Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI Sorbents.

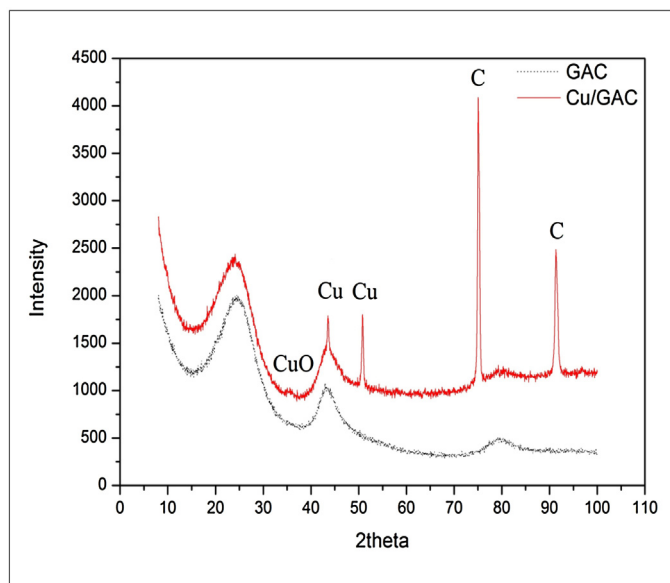


Fig. 3. XRD Patterns of GAC (Dotted-line) and the Cu/GAC Sorbent (Solid-line).

[8]. The diffraction pattern of the Zn/CLI sample does not display any changes as compared to that of the native sorbent (Fig. 6). Similar observations in the structural changes of the Cu^{2+} - and Zn^{2+} ions adsorbed on the inorganic materials have already been reported in previous studies [38].

XPS analysis

The XPS spectra taken from the Cu/GAC sample are presented in Fig. 7. The survey spectrum in Fig. 7a plots the positions of the main peaks characteristic for the main elements from which the sample was composed (*i.e.*, of oxygen, copper, and carbon). The atomic percentages of each component are provided in the inserted Table. The sample consisted mainly of carbon at an atomic percentage of 95.6%, as well as 4.1% of oxygen, and only 0.1% of copper. Despite the small amount of copper in the sample, the fitted high resolution XPS spectrum of the Cu 2p region, as given in Fig. 7b, clearly shows distinct Cu 2p photoelectron lines. The Cu $2p_{3/2}$

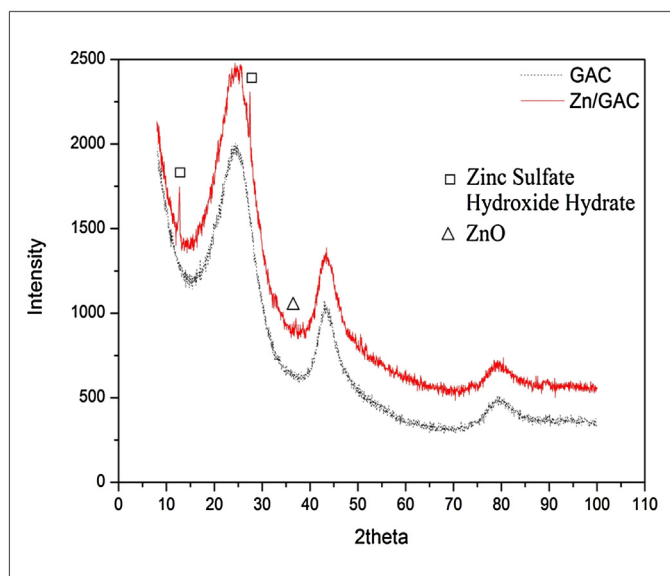


Fig. 4. XRD Pattern of GAC (Dotted-line) and the Zn/GAC Sorbent (Solid-line).

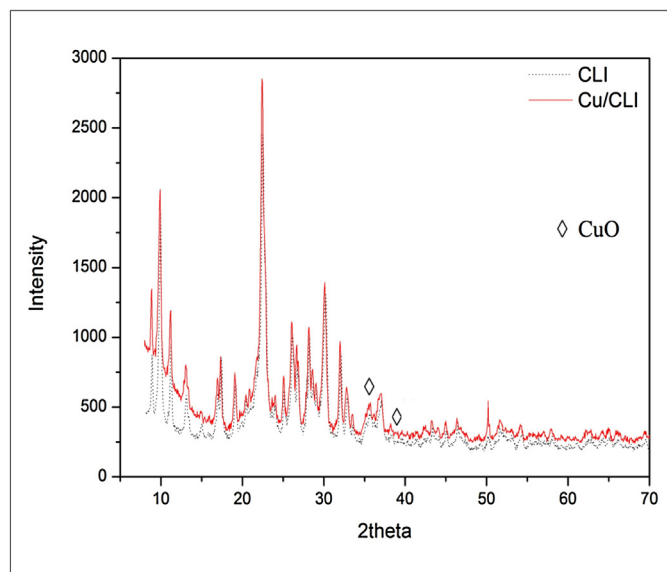


Fig. 5. XRD Patterns of CLI (Dotted-line) and the Cu/CLI Sorbent (Solid-line).

photoelectron line positioned at 935.5 eV corresponds to either CuSO_4 [19], or CuO [39], while the Cu $2p_{3/2,\text{sat}}$ line positioned at 943.1 eV corresponds to CuO [39]. The Cu $2p_{1/2}$ photoelectron line positioned at 953.7 eV additionally confirms the presence of CuO in the sample [40].

The XPS spectra taken from the Zn/GAC sample are plotted in Fig. 8. The survey spectrum in Fig. 8a shows the positions of oxygen, zinc, and carbon. The sample consisted mainly of carbon, at an atomic percentage of 95.99% and 3.95 At.% of oxygen (inserted Table). Although the sample contained only 0.06 At.% of zinc, the position of Zn 2p photoelectron lines could be clearly resolved from the fitted high resolution region spectra given in Fig. 8b. According to literature data [39,41], both the Zn $2p_{3/2}$ and $2p_{1/2}$ lines, positioned at 1022.7 and 1045.8 eV, respectively, indicate that zinc is present in its oxidized state as Zn^{2+} .

The XPS spectra from the Cu/CLI sample are given in Fig. 9. The survey spectrum in Fig. 9a lays out the photoelectron lines of the main elements, Al, Si, O, C, and Cu, as well as their atomic

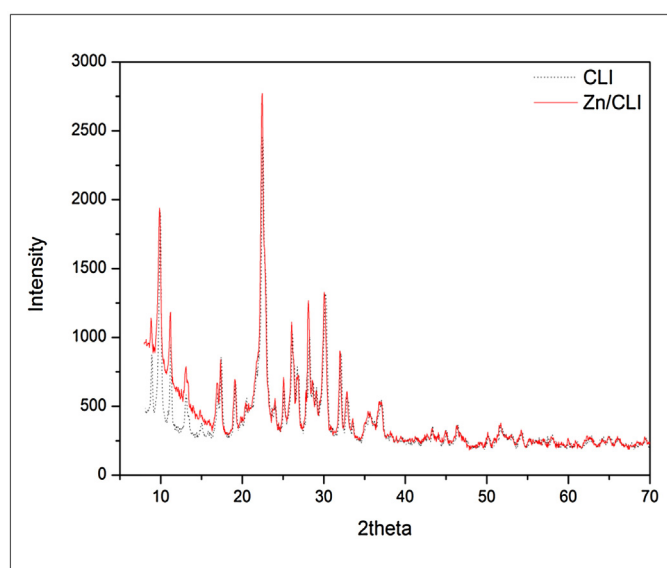


Fig. 6. XRD Patterns of CLI (Dotted-line) and the Zn/CLI Sorbent (Solid-line).

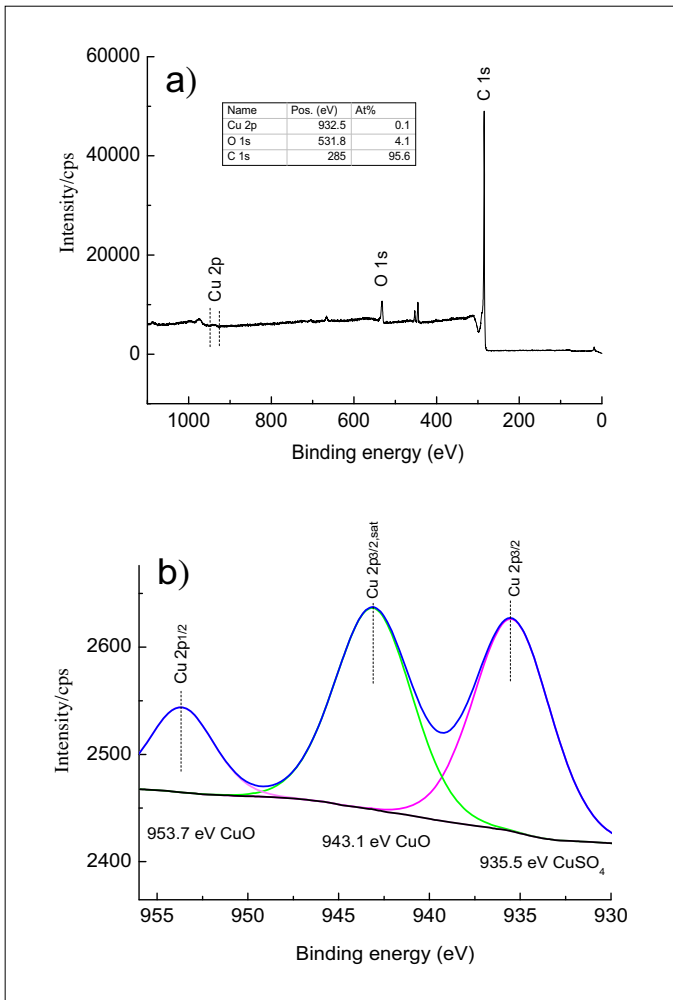


Fig. 7. XPS Spectra Taken from the Cu/GAC Sample: a) the survey spectrum showing the position and the atomic percentage of the main components; b) the high resolution region spectrum of Cu 2p showing the fitted binding energies.

percentages in the inserted Table. The atomic percentage of copper was found to be 0.14%, and the region spectrum resulted in the Cu 2p lines as given in Fig. 9b. The Cu 2p lines were fitted to two contributions both attributed to CuO, i.e., Cu 2p_{3/2}, positioned at 933.7 eV [39], and Cu 2p_{1/2} at 953.6 eV [40].

The XPS spectra taken from the Zn/CLI sample are presented in Fig. 10. The Survey spectrum in Fig. 10a charts the positions of the main elements and their atomic percentage in the inserted Table. The Zn 2p photoelectron lines in Fig. 10b can be confirmed by the fitted high resolution region spectra, from which both Zn 2p_{3/2} and 2p_{1/2} lines positioned at 1022.8 and 1046.0 eV, respectively, indicate the presence of Zn²⁺ ions in the Zn/CLI sample [39,41].

Antimicrobial activity of the Cu²⁺- and Zn²⁺-activated sorbents

The antimicrobial activity of the sorbents activated by the researched metal-ions has been shown to be the result of their reaction loaded onto the surface of the sorbent. Metal activated sorbents tend to attract negatively charged membranes of the bacteria to the surface of the material, where the positively charged metal ions inactivate microbial cells or enable them to replicate [5,6,12]. The low desorption rates of Cu²⁺- and Zn²⁺- ions from activated sorbents of this study allowed for antimicrobial experiments in which only the Cu²⁺- and Zn²⁺-activated surface sites were used (and not their detached ions in the aqueous

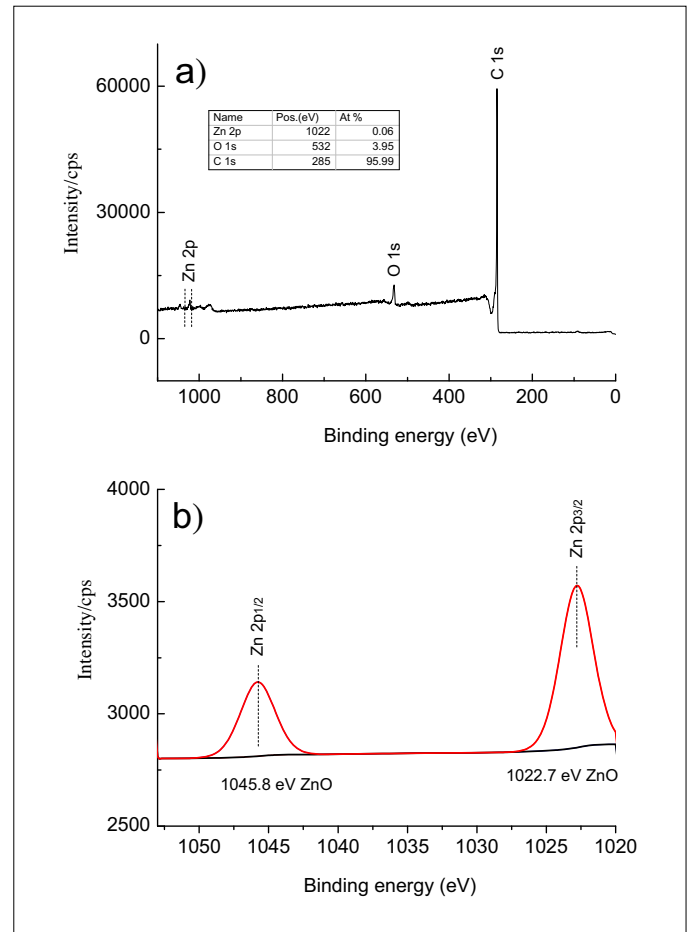


Fig. 8. The XPS Spectra Taken from the Zn/GAC Sample: a) the survey spectrum showing the position and the atomic percentage of the main components; b) the high resolution region spectrum of Zn 2p showing the fitted binding energies.

solutions). Hu et al. have concluded that the antimicrobial activity of Cu-modified montmorillonite manifests in a greater domination of surface particles than by Cu²⁺- ions, owing to the density of Cu²⁺- ions located on the mineral's surface being much higher than the concentration of Cu²⁺- ions released into the solution [25]. The low concentration of desorbed metal ions may indicate that the antimicrobial effect is attributable to the metal ions being immobilized on the surface (i.e., activated sites are responsible for the microbial cell reduction). The results confirming the mechanism of antimicrobial activity of the Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI activated sorbents are presented in Table 3.

The antimicrobial activity of the Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI was tested after 24 h of initially carrying out the activation process, when the maximum Cu²⁺- and Zn²⁺-loadings were obtained. The initial and final pH for each microbial solution was not significantly changed due to the short contact time between the metal-activated sorbent and the microbial solution. The exposure period was limited to 15 min as to minimize the desorption of Cu²⁺- and Zn²⁺- ions into the solution. The low desorption rates of Cu²⁺- and Zn²⁺- ions ensured that the antimicrobial activity of free ions released from the metal-activated surface would be disregarded. For these samples, their contribution of free metal ions in cell removal was less expressed and most of their antimicrobial activity depended on the effect of their metal-activated surface sites. In order to stimulate the maximum reduction of both bacteria and yeast cells, the experiments were performed under daylight. This is in line with earlier

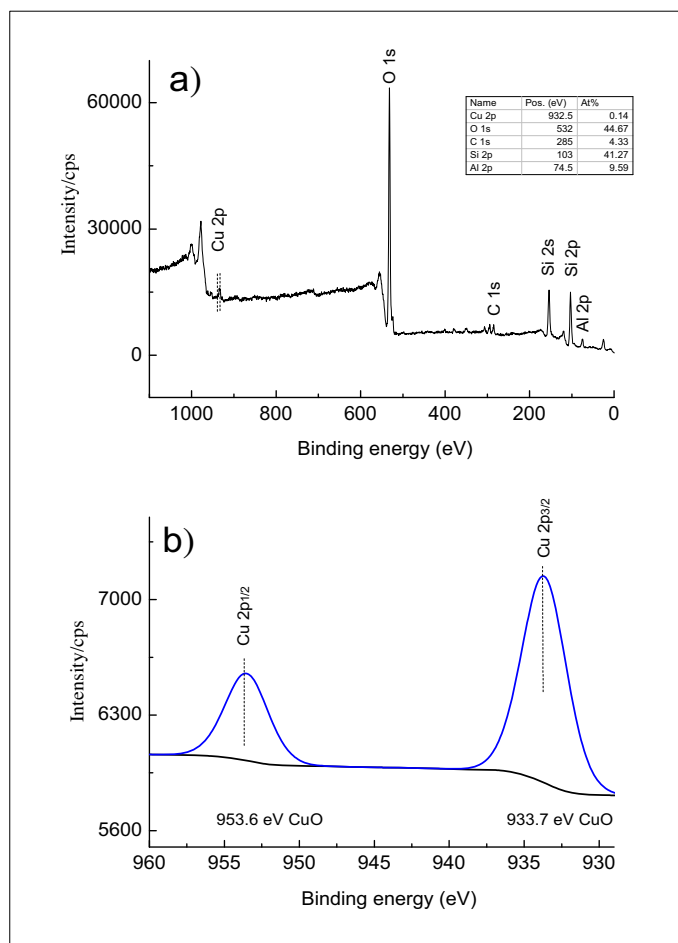


Fig. 9. The XPS Spectra Taken from the Cu/CLI Sample: a) the survey spectrum showing the position and the atomic percentage of the main components; b) the high resolution region spectrum of Cu 2p showing the fitted binding energies.

reports that the influence of light increases the percentage of cell removal [16].

In previous antimicrobial research undertaken on Ag⁺-activated sorbents, samples were tested against microbial cells after desorption tests [13]. Moreover, the microbiological experiments were performed for the activated sorbents in different stages of their reactive exhaustion, e.g. as regarding their different residual metal loadings on the surface of the material [13]. This study concluded that an overall antimicrobial activity of metal-activated sorbent used in different stage of their exhaustion, was primarily a result of the effect of metal ions immobilized on the surface sites (ions from the solid phase). In the investigation of Cu²⁺- and Zn²⁺-activated sorbents, the sorbents were tested after 24 h of activation process, with maximum loadings of Cu²⁺ – and Zn²⁺-ions. The choice of these experimental parameters was already confirmed

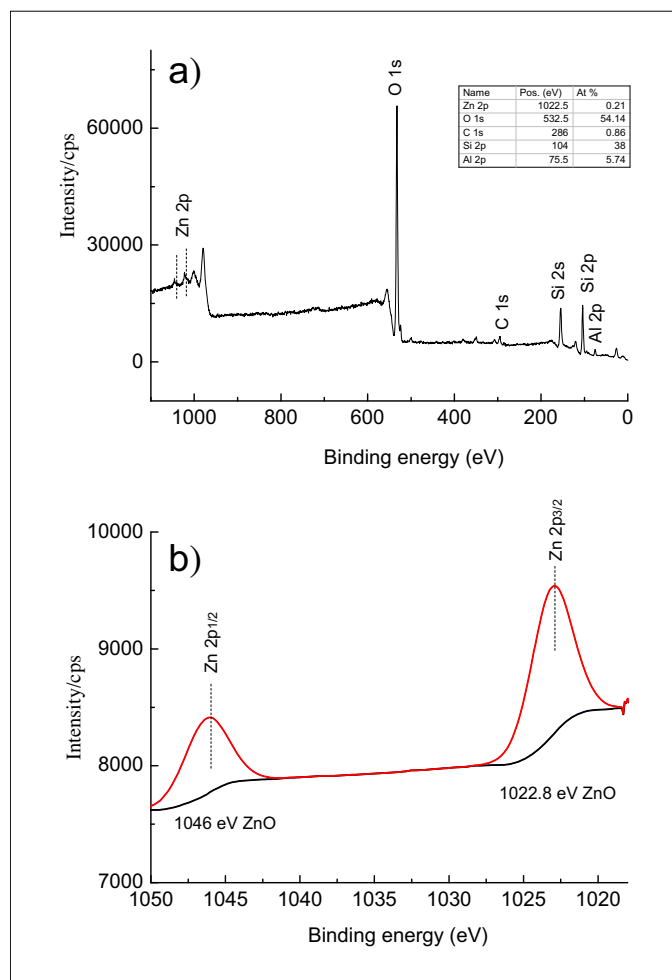


Fig. 10. The XPS Spectra Taken from the Zn/CLI Sample: a) the survey spectrum showing the position and the atomic percentage of the main components; b) the high resolution region spectrum of Zn 2p showing the fitted binding energies.

through the dominant influence of metal ions from the solid phase [13], due to which the focus of this research was to determine the antimicrobial mechanism of the activated surface sites. While the desorption of bacteria from the activated surface was not included in this particular investigation due to it being beyond the exact scope of the research, it merits further investigation as the results of this study show it as a promising area for further research into anti-microbial properties of metal ions.

When the antimicrobial data of the activated sorbents, as given in Table 3, were analyzed, the microbial cell reduction for the Cu²⁺-activated sorbents was found to be effective in the following (ascending) order from *C. albicans*, *S. aureus* to *E. coli*, whereas for the Zn²⁺-activated sorbents, the most resistant microbes were *S. aureus*, *C. albicans* and then *E. coli*. The greatest inhibition rate after

Table 3
The Antimicrobial Activity of the Cu/GAC, Zn/GAC, Cu/CLI and Zn/CLI Activated Sorbents after 15 Min of Exposure to the Microbial Solutions.

Sample	<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>	
	CFU/ml	R (%)	CFU/ml	R (%)	CFU/ml	R (%)
GAC control	3.98×10^5		6.00×10^5		2.20×10^5	
Cu/GAC	7.00×10^3	98.24 ± 0.2	3.00×10^5	50.00 ± 0.3	1.48×10^5	32.73 ± 0.3
Zn/GAC	4.50×10^4	88.69 ± 0.1	5.80×10^5	3.33 ± 0.4	1.34×10^5	39.09 ± 0.2
CLI control	3.83×10^5		5.40×10^5		2.78×10^5	
Cu/CLI	0.00	100.00 ± 0.2	2.20×10^5	59.26 ± 0.2	1.55×10^5	44.24 ± 0.4
Zn/CLI	9.10×10^4	76.24 ± 0.3	5.10×10^5	5.56 ± 0.5	1.84×10^5	33.81 ± 0.3

15 min of exposure was obtained for Cu/CLI and Cu/GAC vs. *E. coli*, at 100.0% and 98.24%, respectively, while for Zn/GAC and Zn/CLI, the cell removal reached 88.69% and 76.24%, respectively. When testing the activated Cu/GAC and Cu/CLI sorbents vs. *S. aureus*, the results showed intermediate inhibition – at 50.0% and 59.26%, respectively – while for Zn/GAC and Zn/CLI, cell reduction was minimal (at 3.33% and 5.56%, respectively). The most coherent cell removal was obtained for *C. albicans*, baring values within the range of 32.73% to 44.24% for Cu/GAC and Cu/CLI, respectively.

When comparing the copper- and zinc-modified materials, it is clear that a more efficient removal method was obtained by the Cu²⁺-activated sorbents (with the exception of Cu/GAC vs. *C. albicans*). This is in agreement with previous studies where *E. coli* and *S. aureus* showed higher sensitivity upon exposure to copper, especially to Cu²⁺- ions [4–6,16]. The antibacterial activity of elemental copper has been previously investigated by Santo et al. [20]. In the present study, Cu/GAC supported by elemental copper (Cu⁰) and a low amount of the ionic form of copper (Cu²⁺) had significant effects for the cell removal of the *E. coli* strain. The low inhibition rate for Zn/GAC and Zn/CLI against *S. aureus* is explicable by the low concentration of Zn²⁺- ions loaded on the activated sorbent (due to its low sorption capacity) which may potentially stimulate bacterial growth since zinc is an essential element [4]. Varying microbial species utilize a range of species specific protective mechanisms when exposed to chemical agents. The sensitivity of various bacteria cells to Cu²⁺- and Zn²⁺- ions have been already reported in numerous other studies [42]; therein, our study has found that the inhibition rate for *C. albicans* was insufficient for all the activated sorbents. Similar results were demonstrated for the antimicrobial activity of Ag⁺-activated GAC and CLI [13]. Linares et al. indicated that *C. albicans* cells are able to produce an anti-oxidation enzyme, *catalase*, which plays a role in protecting the cell from degradation caused by chemical agents [43].

On analyzing the average inhibition rate of the selected GAC and CLI, the results of our study suggest that clinoptilolite is a more efficient activated material. The highest inhibition rate was evidenced by the Cu/CLI activated sorbent against all the tested microbial strains. Since clinoptilolite had the lowest sorption capacity for Cu²⁺-ions as compared to all other metal/sorbent combinations (see Table 1.), the concentration proved not only to be a key factor of the activating agent, but also the accessibility and distribution of the Cu²⁺- ions. These two latter factors led to the most efficient antimicrobial activity of Cu/CLI. This is in accord with previous studies which demonstrate that an increased amount of copper loaded onto the zeolite surface does not result in a significant change in the corresponding antimicrobial activity [16]. The antimicrobial activity of the Cu/CLI sorbent is affected by the presence of Cu²⁺- ions that are incorporated and dispersed into the material, proving that the structural properties of the activated sorbents bare substantial effect on their antimicrobial activity.

When incorporating and comparing all the results of this present study with those of prior investigations, there is considerably significant evidence that the overall antimicrobial effect is not merely related to the presence and quantity of metal ions, but is affected by the surface properties of the materials as well.

Conclusion

In this study, Cu²⁺- and Zn²⁺-activated sorbents were examined in antimicrobial tests against *E. coli*, *S. aureus* and *C. albicans*. The highest inhibition rates, after 15 min of exposure, were obtained for Cu/CLI and Cu/GAC vs. *E. coli*, 100.0 and 98.24%, respectively, while for Zn/GAC and Zn/CLI, cell removal reached 88.69 and 76.24%, respectively. The microbial cell reduction for

Cu²⁺-activated sorbents increased from *C. albicans*, to *S. aureus* to *E. coli*, while for the Zn²⁺-activated sorbents, the most resistant microbes were found to be *S. aureus*, followed by *C. albicans* and *E. coli*. Structural analysis of the activated sorbents (XRD, XPS and SEM analyses) shed light on the chemical state of the activating agents (Cu²⁺- and Zn²⁺- ions) and confirmed the presence of immobilized Cu²⁺- and Zn²⁺- ions, reflecting their antimicrobial efficacy. The pathways of antimicrobial activity have been of specific interest. The antimicrobial activity of the activated sorbents was observed through the inhibition rate of the metal ions loaded onto the surface activated sites. The results indicate that the antimicrobial performance of the activated sorbents depends not only on the concentration of the activating agent, but also on the surface characteristics of the material, as they directly determine the distribution and the accessibility of the activating agent.

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