
Water decontamination by silver and copper montmorillonite

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Abstract: Several bactericide inorganic materials have been used for the purification of water with varying success. The characterisation of the incorporation of Ag or Cu cations in montmorillonite (MMT) clay, to obtain a bactericide material against *E. coli*, indicated that both outer and interlayer surfaces act as adsorption sites of AgO and Ag₂O or CuO, respectively. The Ag-exchanged clays retain their bactericide ability even after being stirred in aqueous suspension, whereas samples with the highest concentration of Cu do not inhibit bacterial growth. Experiences from supernatants of Cu-exchanged clays indicated that bactericide capacity was present at high clay concentration.

Keywords: silver montmorillonite; copper montmorillonite; bactericidal effect.

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Jose Pascual Cosp holds a PhD in Chemistry. He is a Professor at the School of Industrial Engineering at the University of Málaga. His research interest focuses water treatment with different methods including the use of clays. He has published one patent, more than 60 papers in international scientific journals and more than 50 participations in conferences. He has directed more than 36 research projects funded by private and public institutions.

Sofia de la Fournière graduated Biology from the University of Buenos Aires. Her undergraduate final thesis focused on the biocidal effect of modified clays to purify water in rural communities. She has published two papers and has presented four papers at national and international conferences. She is a member of the research group on medical and environmental parasitology at the Department of Biological Chemistry, Faculty of Exact and Natural Sciences, University of Buenos Aires.

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1 Introduction

Inorganic bactericidal materials have advantages over traditional organic ones, mainly owing to their chemical stability, heat resistance, secure application, long period of action, etc. (Ohashi et al., 1998). In general, inorganic materials are based on the use of metal ions that have bactericidal properties such as Ag^+ or Cu^{2+} , loaded into a ceramic matrix by ion exchange (Malachová et al., 2011; Guerra et al., 2012).

Clays, zeolites (Top and Semra, 2004) and synthetic aluminosilicates have been used to support these ions, with good results (Magaña et al., 2008). In particular, the characteristics of MMT clays such as high cation exchange capacity (120 meq/100g), specific surface (700 m^2/g), negative surface charge in a wide pH range (Avena and De Pauli, 1998), chemical inertia (Carretero, 2002) and non-toxicity (Viseras et al., 2007) enable their utilisation as adsorbent.

MMT belongs to the smectite group, and has a layered structure (1 : 2: Al : Si) with isomorphic substitutions (Al^{3+} by Si^{4+} , Fe^{3+} and Mg^{2+} and Al^{3+}), which generate negative electrical charges in the crystal structure. These negative structural charges are compensated by the presence of hydrated metal cations (Na^+ , K^+ , Ca^{2+} , etc.) in the interlayer, which can be exchanged with other cations, such as metal cations. This latter feature, which is given by the large exchange capacity, is the driving force for the surface retention of Ag^+ and Cu^{2+} .

The bactericidal activity of Ag ion was assigned to different behaviours:

- affinity of Ag^+ for sulfhydryl groups (-S-H) exposed on the bacterial surface that form a (-S-Ag) stable bond capable of inhibiting the energy transfer system
- catalysing silver from the reaction of coupling sulphur atoms to form R-S-S-R bonds that disrupt cellular respiration and electron transfer (Davies and Etris, 1997)
- electrostatic attraction of Ag^+ favoured by carboxylic groups present in certain bacteria walls, affecting nucleic acids and cytoplasmic proteins, disrupting essential cell functions or inhibiting cell replication (Feng et al., 2000)

- moreover, Ag⁺ can inhibit bacterial DNA replication by a mechanism involving Ag insertion between purines and pyrimidines and the subsequent rupture of hydrogen bonds (Klueh et al., 2000).

Numerous microorganisms have developed regulatory systems to control the capture, extrusion or oxidation of the metal ion (Espariz et al., 2007).

In the case of Cu, it was suggested that:

- The decreased use of oxygen by *E. coli* injured by copper is associated with damage in the respiratory chain (Domek et al., 1984).
- Moreover, scanning electron microscopy reveals the formation of cavities or pits on the *E. coli* surface as a result of the adhesion of copper nanoparticles to the bacteria wall because of their opposite electrical charges (Raffi et al., 2010).

The importance of the exchange of both Ag and Cu cations lies in their antibacterial or antifungal properties (Malachová et al., 2011), which are transmitted to the system Ag-MMT and Cu-MMT. Usually, antibacterial compounds are effective when they are released from the inorganic support (Malachová et al., 2009) allowing their use in water decontamination.

The purpose of this paper is to characterise one-metal systems, Ag-MMT and Cu-MMT, and two-metal systems, Ag/Cu-MMT, keeping in mind the possibility of their technological application in water decontamination.

2 Materials and methods

2.1 Materials

A bentonite (MMT) sample from Argentine North Patagonia (Rio Negro) was used as raw material.

The main properties and mineralogy of this clay were determined in previous work (Magnoli et al., 2008) and are summarised in Table 1.

The chemical analysis, performed on a purified sample of MMT, indicated a highly charged smectite (0.41 eq/formula unit) with a structural formula: [(Si_{3.89} Al_{0.11}) (Al_{1.43} Fe_{0.26} Mg_{0.30})] M⁺_{0.41} (Magnoli et al., 2008). M⁺ indicated the presence of Na⁺ (1.39% as Na₂O) and Ca²⁺ (2.31% as CaO) content in the interlayer (Magnoli et al., 2008).

Table 1 Cationic exchange capacity (CEC), isoelectric point (IEP), external specific surface area (determined by N₂ adsorption, BET method; SN₂) and mineralogy of MMT sample

Sample	CEC (meq/100g)	IEP (pH)	SN ₂ (m ² /g)	Mont. (%)	Quartz (%)	Feldspar (%)
MMT	174	2.7	34.0	84	12	4

2.2 Synthesis of Ag- and Cu-exchanged samples

The synthesis of Ag or Cu-MMT samples was performed by the following procedure: 10 g of MMT was dispersed in 1 L of deionised water to which the corresponding amount

of powder salt to attain 0.1 M AgNO₃ or 0.1 M CuSO₄ was added. The reaction mixtures were stirred overnight at room temperature. The products were washed successively with distilled water by centrifugation to free them of anions (tested by AgNO₃ or BaCl₂, respectively), dried at 80°C and ground in an agate mortar.

Mixed exchanged samples (Ag and Cu) were obtained from Ag-MMT suspension to which CuSO₄ powder was added to attain 10⁻³ M (Ag/Cu10⁻³-MMT) or 10⁻² M (Ag/Cu10⁻²-MMT), and the same washing procedure as indicated previously for Ag-MMT was used.

2.3 Materials characterisation

All samples were characterised by X-ray diffraction (XRD) in Philips PW 3710 equipment with CuK_α radiation and Ni filter at 40 kV and 30 mA in oriented samples. They were prepared by spreading the sample suspension on glass slides, followed by drying at room temperature for 48 h and with atmospheric humidity control $rh = 0.47$.

The total specific surface area (TSSA) was determined by water vapour adsorption (Torres Sánchez and Falasca, 1997).

The electrophoretic mobility was performed in 1% suspension samples by microelectrophoresis using Brookhaven 90Plus/Bi-MAS equipment in the zeta potential function using 10⁻³ M KCl as inert electrolyte and Pd electrodes.

The X-ray photoelectron spectroscopy (XPS) capability to determine the oxidation status of Ag and Cu exchanged on MMT was used performing the XPS analyses on a Physical Electronics PHI 5700 spectrometer with non-monochromatic Mg K α radiation (300 W, 15 kV, 1253.6 eV).

The sample powders were pressed into small stainless steel troughs of inner diameter 4 mm and 0.5 mm depth, mounted on a multispecimen holder. The pressure in the analysis chamber was about 10⁻⁷ Pa. The following sequence of spectra was recorded: survey spectrum, C1s, O1s; Na1s; Mg2p, Al2p, Si2p, Ca2p; Fe2p; Cu2p; Ag3d and C1s again to check for charge stability as a function of time and the absence of degradation of the sample during the analyses. The C-(C, H) component of the C1s peak of carbon was fixed at 284.8 eV to set the binding energy scale.

The rate applied to correct the charging effect was between 2.8 and 3.5 eV (Moulder et al., 1992). Analysis software PHI ACCESS ESCA-V6.0 F was used for data acquisition and a time period of 10 min was utilised for Cu2p Ag3d analysis to prevent the photoreduction of Ag⁺ and Cu²⁺ species. However, the reduction of Cu²⁺ in high vacuum cannot be excluded from the analysis (Poulston et al., 1996). Molar fractions were calculated using peak areas normalised on the basis of acquisition parameters after a linear background subtraction, experimental sensitivity factors and transmission factors provided by the manufacturer.

Ag and Cu desorption was analysed in suspension (2% w/w) of all samples, stirred for 48 h in river water (simulated) (Webber and Thurman, 1991) and deionised water solutions. The supernatants of the suspensions were centrifuged at 25,000 rpm for a period of 20 min and analysed by AA Ag and Cu.

3 Determination of the bactericidal capacity of clays

To evaluate the effect of modified clays on the viability of microorganisms, bacteria were cultured in liquid medium to which either preincubated clays in aqueous suspension or the aqueous supernatant were added. *Escherichia coli* Dh5 α strain was incubated in nutrient broth (3 h, 37°C, 250 rpm) and its density was estimated by counting colony forming units (CFUs) after incubation in solid nutrient agar (24 h, 37°C). The final working density was adjusted to 6×10^3 CFU/mL by properly diluting the microorganism suspension to enable the development of colonies in the range 30–300 CFU.

Clays were sterilised by exposure to UV radiation for 30 min and suspended in sterile dechlorinated tap water (0.2% for MMT, Ag-MMT, Ag/Cu 10^{-3} -MMT, Ag/Cu 10^{-2} -MMT; 0.2 and 2% for Cu-MMT) and incubated under continuous stirring (2 h, 29°C, 250 rpm). Then, the clay slurry was transferred to a syringe attached to a sterile filtering device equipped with a mixed cellulose ester membrane (Millipore®, pore diameter 0.45 μ m). After applying positive pressure through the plunger, the device was disassembled and membranes with trapped solids were carefully removed and introduced into conical flasks containing 25 mL of bacterial suspension in nutrient broth (1 : 2). Sterile filtrates were incubated with bacterial suspension (1 : 1), so as to match the bacterial density in the incubations of the solid phase.

These new suspensions were incubated (2 h, 29°C, 250 rpm) and finally, 50 μ L aliquots were plated in quadruplicate on nutrient agar and incubated for 24 h at 37°C prior to performing the CFU counts.

A series of controls were included:

- positive control for bacterial development (100% growth)
- negative control (nutrient broth)
- copper solution controls prepared by adding a volume of 0.1 M CuSO $_4$ (sterilised through a Millipore® membrane, pore diameter 0.45 μ m) containing the number of Cu equivalents provided by a 2% Cu-MMT suspension.

The second copper control was a 1 : 100 dilution of the former. Controls were submitted to the same experimental method already described.

4 Results

As was indicated previously (Fernández et al., 2013), the specific interactions between N $_2$ (as probe molecule) and the polar interlayer surface of MMT do not allow an accurate determination of the specific surface area of expandable clay minerals (MMT). The possibility that the water molecule enters the interlayer enables its adsorption to include this inner surface and therefore, determine the TSSA.

The TSSA values of the indicated samples are summarised in Table 2. The TSSA values for samples with Cu and Ag showed a decrease and an increase, respectively, with respect to the MMT sample. These results reflect the different complex formation between the respective cations and the MMT surface. The Cu $^{2+}$ layout in the outer and interlayer MMT surfaces corresponds to an internal sphere complex (Hu and Xia, 2006; Fang et al., 2010), decreasing the TSSA value obtained compared with that of the MMT

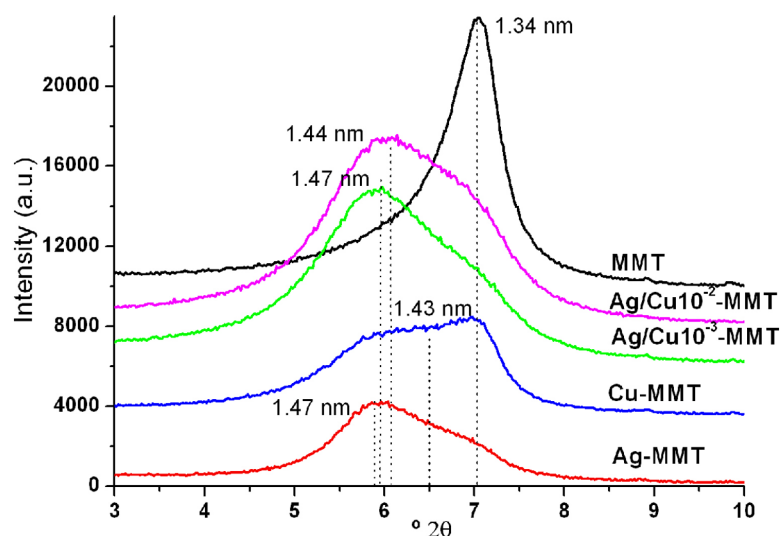
sample. However, the greater water adsorption for the Ag-MMT sample could indicate the formation of a majority outer-sphere complex.

Table 2 TSSA of indicated samples

Sample	TSSA (m ² g ⁻¹)
MMT	573
Ag-MMT	677
Cu-MMT	236
Ag/Cu10 ⁻³ -MMT	260
Ag/Cu10 ⁻² -MMT	253

Figure 1 shows the partial XRD spectrum of the (001) reflection peak for the indicated samples. The Ag-MMT and Cu-MMT samples showed the respective cation incorporation in the MMT interlayer through the increase of the (001) reflection peak (1.47 and 1.43 nm, respectively) with respect to that of the MMT sample (1.34 nm). These variations of the 001 value and also the reflection peak asymmetry reflected water molecule retention in different proportions caused by the different cations present in the interlayer of each sample. The Ag/Cu10⁻²-MMT and Ag/Cu10⁻³-MMT samples generated (001) reflection peaks of 1.47 and 1.44 nm, respectively, which are values intermediate to those found for Ag-Cu-MMT and MMT samples.

Figure 1 X-ray diffraction patterns of oriented samples (see online version for colours)



The 001 reflection peak asymmetry for all samples indicated the existence of a heterogeneous interlayer width, originated by the different ratio of the cation exchanged and water molecule amounts present in the interlayer. To determine the composition of the reflection peaks (Figure 1), a mathematical deconvolution was performed and the results are summarised in Table 3.

The 001 reflection peak for the MMT sample was deconvoluted into two peaks at 1.26 and 1.34 nm, respectively, assigned to a water monolayer and almost a bilayer (1.47 nm, Ferrage et al., 2005) related to the Na⁺ and Ca²⁺ content (Magnoli et al., 2008), as was indicated by the ratio obtained by the chemical analysis, CaO/Na₂O = 1.66.

Table 3 001 reflection peak deconvolution, from Figure 1

<i>Sample</i>	(nm)	<i>Area</i>	(nm)	<i>Area</i>	<i>Area ratio</i>
MMT	1.34	3782	1.26	2716	1.4
Ag-MMT	1.47	5050	1.26	886	5.7
Cu-MMT	1.43	7683	1.26	1450	5.3
Ag/Cu10 ⁻³ -MMT	1.47	16,381	1.26	500	32.7
Ag/Cu10 ⁻² -MMT	1.44	17,316	1.24	676	25.6

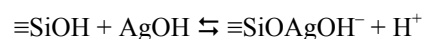
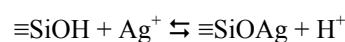
The mathematical deconvolution (Table 3) indicated the presence of two peaks for all cation-exchanged samples.

The 001 reflection peak at values close to 1.26 nm corresponded to cations associated with a monolayer of water molecules. This association has been described previously by Oueslati et al. (2006) and Zhou et al. (2008). Particularly in the Ag/Cu10⁻²-MMT sample, a smaller value of this peak (1.24 nm) was observed, which may be due to the fact that the presence of both cations (Cu and Ag) generated lower water content in the interlayer.

The 001 reflection peak found close to 1.5 nm could be assigned to the presence of hexa-aquo (Cu [H₂O]₆⁺²) (He et al., 2001) or tetra-aquo (Ag[H₂O]₄⁺) (Ohtaki and Radnai, 1993) complexes or to the high ionic radii of Cu⁺² and Ag⁺ (0.70 and 0.89 Å, respectively; Ball and Norbury, 1974). This 001 reflection peak for the Ag/Cu10⁻²-MMT sample (1.44 nm) was nearer to that of the Cu-MMT than to that of the Ag-MMT sample. This behaviour could be assigned to a displacement of the cation Ag⁺ by the presence of Cu⁺² in the interlayer, in agreement with the Schulze-Hardy rule for cation displacement (Swartzen-Allen and Matijevic, 1997) and is consistent with the amount of Cu⁺² and Ag⁺ determined subsequently by XPS measurements (Table 5).

The influence on the external surface of the clay content by different metal cations may be evidenced by zeta potential measurements as a function of pH (Thomas et al., 1999). The curves of zeta potential for all samples are shown in Figure 2. For the MMT sample, the flat negative zeta potential curve found in all the investigated pH values, as was indicated previously (Fernández et al., 2013), was generated by the predominance of negative charges on the faces of the particles compared with the positive charge on the edges. The exchanged samples showed more negative values of zeta potential curves (within -35 to -45 mV) throughout the pH range 3–7 than that of the MMT sample (-30 mV).

Different reactions may be responsible for the increase in surface negative electric charge of the exchanged samples. First, a reduction of the cation oxidation state (Ag⁺ and Cu²⁺ to Ag⁰ or Cu⁺, respectively) on the MMT surface could occur and, consequently, the negative surface charge is not compensated as in the presence of natural cations (Na⁺ and Ca²⁺). Second, as was indicated by Shen et al. (2013), owing to their empty orbital, silicon surface groups hydroxylated in water and silver ions have a strong tendency to react by surface coordination with the previous hydroxylated Si surfaces, according to the following equations:



The last reaction could produce the increase in negative charge found in the exchanged Ag-MMT, Ag/Cu10⁻²-MMT and Ag/Cu10⁻³-MMT samples.

The Cu-MMT and Ag/Cu10⁻²-MMT samples showed a decrease in negative charge at alkaline pH (greater than pH = 7), with respect to those of the MMT sample. Copper ions could follow similar behaviour of surface coordination, decreasing the zeta potential as indicated for silver ions at low pH, whereas when the pH is greater than 6, precipitation of copper occurs in the form of hydroxide and the negative zeta potential of the surface decreases (Erdemoğlu and Sarikaya, 2006; Doğan et al., 2009).

The atomic concentration values, corrected by the RSF factor, obtained from XPS are listed in Table 4.

Figure 2 Zeta potential against pH curves of all samples. Symbols indicate: (●) Ag/Cu10⁻³-MMT; (●) Ag/Cu10⁻²-MMT; (■) MMT, (▲) Cu-MMT and (■) Ag-MMT (see online version for colours)

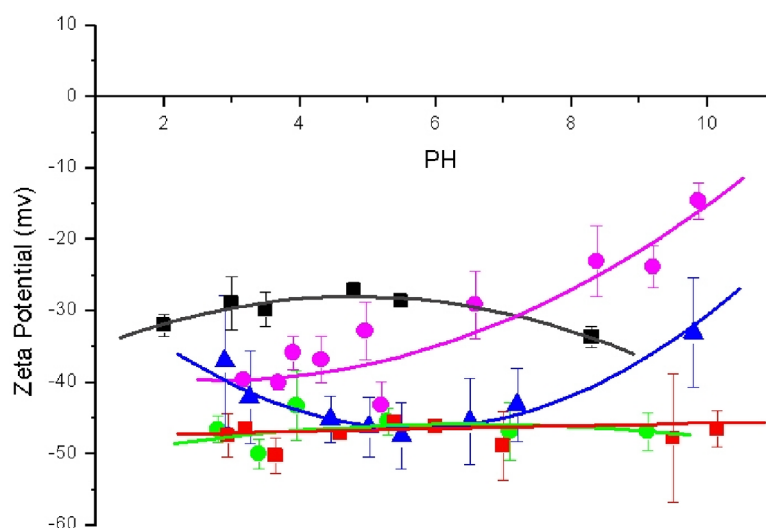


Table 4 Atomic concentration values, corrected by the RSF factor, obtained from XPS

Sample	C1s	O1s	Na1s	Mg2p	Al2p	Si2p	Ca2p	Fe2p	Ag3d	Cu2p
Ag-MMT	6.04	6.01	0.62	1.05	8.63	21.51	0.37	0.88	0.79	–
Cu-MMT	8.66	59.09	1.04	–	9.40	20.49	–	0.64	–	0.67
Ag/Cu10 ⁻³ -MMT	7.10	59.93	0.43	1.17	8.89	20.88	0.31	0.87	0.14	0.27
Ag/Cu10 ⁻² -MMT	8.30	59.04	0.09	1.19	9.45	20.60	0.17	0.64	0.07	0.46

In the Ag-MMT and Cu-MMT samples, the Ag and Cu cation incorporations were evidenced (0.79 = atomic Ag3d and 0.67 = atomic Cu2p concentrations). The raw atomic concentration of Na and Ca (3.35 and 1.08, respectively) was determined by the chemical analysis of the MMT sample (Magnoli et al., 2008). The decrease in atomic concentrations for Na and Ca found in Table 4 corroborates the exchange by Ag and Cu,

respectively. In the mixed (Cu and Ag) samples, the highest value of Cu concentration in both samples revealed a greater surface displacement of Ag^+ by the further addition of Cu^{2+} following the Schulze-Hardy rule for cation displacement, as was indicated previously. The high amount of *O*1s found in samples with exchanged Cu indicated the presence of oxides/hydroxides.

The binding energy of the *Ag3d* doublet could indicate the presence of Ag^+ or Ag^{++} in Ag-exchanged samples. The binding energy values of the *Ag3d* doublet obtained (Figure as supplementary material) were 368.75 and 374.75 eV and 368.82 and 374.28 eV, for Ag-MMT and Ag/Cu10⁻³-MMT samples, respectively. The first of these values are close to those observed in the literature for AgO and Ag₂O (368.6 and 367.8 eV, respectively; Ferraria et al., 2012), while values for Ag^0 were slightly lower (368.0 eV; Cui et al., 2013). Recently, Ferraria et al. (2012) indicated that the peak shape and width can be more useful to indicate the silver oxidation state present, to solve the great discrepancies found in the literature to assign the silver oxidation state from its most intense peak (368 eV). This author indicated that FWHM peak width of 1.12 eV was assigned to Ag^0 , while values between 1.22 and 1.82 eV correspond to Ag^+ and Ag^{++} in Ag₂O or AgO compounds. The FWHM values of 1.83 and 2.09 eV found for Ag MMT and Ag/Cu10⁻³-MMT samples would indicate the main presence of these silver oxidation states on the MMT surface.

In the Cu-MMT sample, the binding energy observed for the *Cu2p* peak was found at 933.30 eV (Figure supplementary material), which is in close agreement with the value of 933.6 eV for CuO, and a shake-up characteristic of Cu^{++} was also observed (Meda and Cerofolini, 2004).

The Cu or Ag desorption analysis in all samples leached with river water or deionised water yielded the values shown in Table 5.

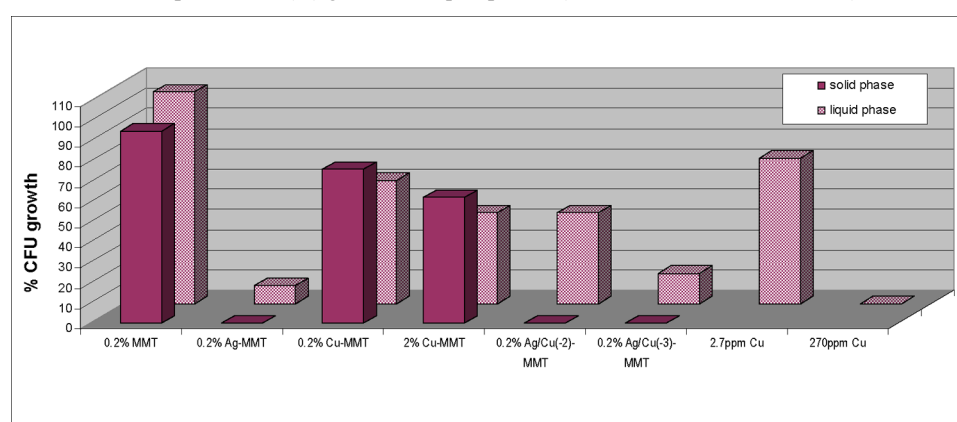
Table 5 Ag and Cu in the supernatants of samples leached with deionised or simulated river water

Sample	Simulated river water		Deionised water	
	Ag (ppm)	Cu (ppm)	Ag (ppm)	Cu (ppm)
Blank reference	0	0.05	0	0.06
Ag-MMT	104	–	101	–
Cu-MMT	–	1	–	5
Ag/Cu10 ⁻² M-MMT	5	14	19	9
Ag/Cu10 ⁻³ M-MMT	49	6	50	6

From Table 5, no significant differences can be seen in the concentration of cations in solution between samples leached with distilled or simulated river water, indicating no significant influence of the cations present in the water. The concentration of Ag cations released into solution from Ag-Cu-MMT samples was lower than the concentration found for the Ag-MMT sample. The lowest concentration observed in the leachate of copper-exchanged samples indicates a strong binding energy in accordance with the formation of inner-sphere complexes with the MMT clay surface. The opposite leaching behaviour of the Ag-MMT sample could indicate outer-sphere complex formation with the MMT clay surface.

The analysis of the biocidal effect of clays was conducted through an experimental method designed to test whether both phases, the solid and the supernatant harvested after stirring a clay slurry, have the ability to alter the growth pattern of a liquid culture of *E. coli*. CFU counts below 50% compared with control cultures were arbitrarily established as a criterion of inhibition of bacterial growth. Figure 3 shows the result of these experiences.

Figure 3 Percentage of CFU development of *E. coli* incubated in the presence of clays. Each bar represents the average of independent experiments performed in quadruplicate and repeated over two to four different days. Results are expressed as percentage of colony development with respect to the control (100%). Symbols are (■) growth in solid phases and (▨) growth in liquid phases (see online version for colours)



First, it was verified that MMT by itself had no effect on bacterial growth since the addition of either the solid or the liquid supernatant to the culture medium allowed us to attain colony counts similar to that obtained in control cultures performed in the absence of clays (CFU growth: MMT solid phase: 95%; MMT liquid phase: 105%, control culture: 100%).

Bacterial growth was inhibited by solid and liquid phases of Ag-MMT slurry (CFU growth: 0 and 9%, respectively). Despite the silver amount released into the solution (Table 5), the Ag concentration in the culture medium was high enough to prevent bacterial growth. Furthermore, the solid still retained its biocidal capacity.

Our results are consistent with those obtained by other authors. The bactericidal effect of Ag-MMT (modified by heating or milling) in solid cultures was ascribed to the silver cation incorporated to the clay (Magaña et al., 2008). In addition, Ag-MMT dispersed in water exhibited a strong antibacterial activity against gram-negative bacteria (Valášková et al., 2010). However, in both cases, the research focused on the assessment of the effect of silver-modified clays disregarding the possibility of migration of the cation from the clay matrix.

When the effect of low amounts of Cu-MMT (0.2%) was analysed, bacterial growth did not differ from the control (CFU growth: 76% for the solid, 61% for the supernatant). A tenfold increase in clay concentration (2% Cu-MMT) reduced the growth of microorganisms only upon addition of the aqueous supernatant (CFU growth: 45% for the liquid phase and 62% for the solid; Figure 3). It has to be noted that stirring 2% Cu-MMT for 48 h allowed the passage of only minimal amounts of Cu (1–5 ppm)

to the solution, indicating that most copper was retained in the solid (Table 5). In our experiments, a methodological detail that could not be avoided was that the highest Cu-MMT concentration settles spontaneously even under continuous stirring at 250 rpm. Therefore, the absence of culture inhibition was more likely caused by the lack of contact between bacteria and the solid. Moreover, assays in this paper were performed with short contact times between bacteria and the solids or their supernatants. Probably, longer contact times would produce a more significant impact on microorganism development.

To test whether copper can inhibit bacterial growth, Cu solutions were added to the culture medium to reach 270 ppm and 2.7 ppm (final concentration). The former was selected given the maximum number of equivalents of metal that can be incorporated into the MMT (1.72 meq/L). Assuming that all of copper could be released from the clay, then the expected Cu peak concentration in a water slurry would be 270 ppm. The lower concentration (2.7 ppm Cu) was chosen within the range of copper released into a 2% aqueous suspension (Table 5). In fact, Figure 3 shows that when bacteria were incubated in the presence of 2.7 ppm Cu, the number of *E. coli* colonies developed was not different from that obtained in control cultures (CFU growth: 72% vs. 100%). However, no colonies developed when cultures were grown in the presence of the highest Cu concentration (CFU growth: 0%).

By contrast, bacterial inoculum incubated with solids containing both cations Ag/Cu(10^{-2})-MMT and Ag/Cu(10^{-3})-MMT showed no colony development, and the addition of the liquid phases caused a marked decrease in growth compared with the control (CFU growth: 45% and 15% for Ag/Cu(10^{-2})-MMT and Ag/Cu(10^{-3})-MMT, respectively). The results suggest a trend in bacterial growth pattern inversely related to the concentration of Ag/Cu ions since the decrease in one order of magnitude of such concentration increases the number of grown colonies (from 15% to 45%; Figure 3). Possibly, the bactericidal effect caused by the supernatant is due to the desorption of silver ions from the clay since Cu-MMT supernatants have virtually no antibacterial power except for those resulting from stirring 2% clay in water.

5 Conclusions

The TSSA values of exchanged samples showed a variation from those of the MMT sample, depending on the cation exchanged and the complex formation with the MMT surface. These variations in water absorption were consistent with the changes in the areas of the 001 reflection peak of XRD, indicating that such adsorption largely takes place on the surface of the interlayer. Changes in the surface charge of the exchanged samples showed that the adsorption of Ag or Cu also occurs on the outer surface of MMT. XPS analysis showed the incorporation of Ag or Cu, with the presence of AgO and Ag₂O or CuO, respectively. The analysis of Ag or Cu desorption indicated a greater release of Ag than Cu in all samples.

All silver-containing clays (Ag-MMT, Ag/Cu-MMT) retained the ability to inhibit microbiological growth even after been stirred in aqueous suspension. By contrast, even the highest concentration of Cu-MMT did not inhibit bacterial growth. Thus, the antibacterial activity of mixed samples (Ag/Cu-MMT) could be attributed to the presence of Ag in the clay.

Desorbed silver from Ag-MMT inhibited colony development. Inhibition of bacterial cultures was also caused by Ag released from clays containing it in combination with Cu. Supernatants from Cu-MMT suspensions were inhibitory when the clay was used in high concentrations.

These results confirm that there was a clear effect of Ag ion on bacterial growth and suggest that the antibacterial power of Cu-MMT depends on the desorption of copper, which determined its concentration in the suspension. Consequently, the application of MMT as biocide will be efficient when using a relatively large concentration of MMT-Cu.

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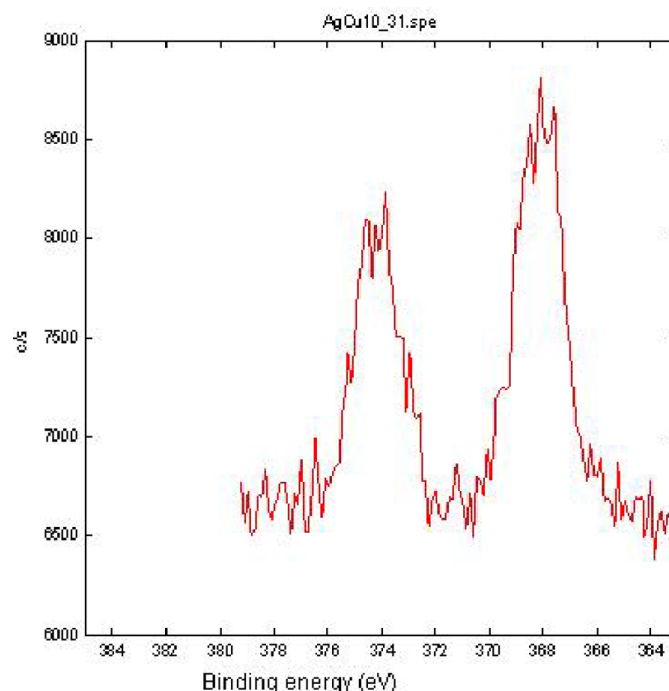
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Supplementary materials

Figure A Ag 3d XPS peak recorded for AgCu10⁻³-MMT sample (see online version for colours)



Supplementary materials (continued)

Figure B Cu2p XPS peak recorded for Cu-MMT sample (see online version for colours)

