



**HAL**  
open science

## Divalent metal release and antimicrobial effects of layered double hydroxides

Jazia Awassa, Damien Cornu, Samantha Soulé, Cédric Carteret, Christian Ruby, Sofiane El-Kirat-Chatel

► **To cite this version:**

Jazia Awassa, Damien Cornu, Samantha Soulé, Cédric Carteret, Christian Ruby, et al.. Divalent metal release and antimicrobial effects of layered double hydroxides. *Applied Clay Science*, 2022, 216, pp.106369. 10.1016/j.clay.2021.106369 . hal-03467360

**HAL Id: hal-03467360**

**<https://hal.science/hal-03467360>**

Submitted on 6 Dec 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 **Divalent Metal Release and Antimicrobial Effects of Layered Double Hydroxides**

2 Jazia Awassa<sup>1</sup>, Damien Cornu<sup>1</sup>, Samantha Soulé<sup>1</sup>, Cédric Carteret<sup>1</sup>,

3 Christian Ruby<sup>1\*</sup>, Sofiane El-Kirat-Chatel<sup>1</sup>

4 <sup>1</sup>Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement

5 (LCPME), Université de Lorraine, 405 Rue de Vandoeuvre, 54600, Villers-lès-Nancy, France

6 \*Corresponding author:

7 Christian Ruby: [Christian.ruby@univ-lorraine.fr](mailto:Christian.ruby@univ-lorraine.fr)

## 8 Abstract

9 Resistance to antimicrobial agents is responsible for major social and economic losses. The  
10 World Health Organization estimated 700,000 global deaths a year due to antimicrobial  
11 resistance. The use of layered double hydroxides (LDHs) as antibacterial materials could present  
12 a way to reduce the risk of bacterial infections and antibacterial resistance, by partial release of  
13 metallic ions in aqueous dispersion. The partial dissolution of different synthetic LDHs  $M^{II}-Al^{III}$   
14 ( $M= Zn, Cu, Ni, Co, Mg$ ) was studied in Lysogeny Broth (LB) and Tryptic Soy Broth (TSB),  
15 growth media of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria,  
16 respectively. The influence of several parameters (crystallinity,  $M^{II}:Al^{III}$  ratio, type of  
17 intercalated anion  $\{CO_3^{2-}, Cl^-, NO_3^-, ClO_4^-\}$  and nature of  $M^{II}$  cations) was investigated. In the  
18 absence of any post-synthetic hydrothermal treatment,  $Zn^{II}-Al^{III}$  LDH showed a release of  $Zn^{II}$   
19 ions 6 times enhanced. Upon increasing  $Zn^{II}:Al^{III}$  molar ratio to 3 : 1 and exchanging carbonate  
20 anions with other anions having lower intercalating affinities, a similar effect was observed . The  
21 dissolution properties of  $M^{II}-Al^{III}$  LDHs were correlated with the thermodynamic stability of  
22 their  $M^{II}(OH)_2$  hydroxide counterparts with the exception of  $Cu^{II}$ -based LDH, which showed an  
23 amplified release of  $Cu^{II}$  due to its irregular structure presenting defects. Finally, the antibacterial  
24 activity was only noted for  $Zn^{II}$  and  $Cu^{II}$ -based LDHs. The antimicrobial effect of the studied  
25 LDHs was linked in the first place to the nature of divalent metal itself, and to the amount of  
26 released  $M^{II}$  ions into the culture media in the second place. This effect was more easily  
27 identified in  $Zn^{II}-Al^{III}$  LDHs whose minimum inhibitory concentration was decreased  
28 significantly from 12 to  $0.375\text{ mg}\cdot\text{mL}^{-1}$  when higher amounts of  $Zn^{II}$  ions were released.

29

- 30 **Keywords**
- 31 LDHs
- 32 Dissolution
- 33 Bacterial inhibition
- 34 Crystallinity
- 35 Zinc
- 36 Copper

## 37 1. Introduction

38 Layered double hydroxides (LDHs) are clay lamellar solids having a structure based on the  
39 stacking of brucite-like sheets comprising divalent and trivalent metal hydroxides. These  
40 sheets hold a net positive charge which is compensated by negatively charged intercalating  
41 anions and water molecules present in the interlaminar regions. The general formula of LDH can  
42 be expressed as:  $[M(II)_{(1-x)} M(III)_x (OH)_2]^{x+} [A^{n-}_{x/n} \cdot yH_2O]^{x-}$ , where M(II) and M(III) are  
43 divalent and trivalent metal cations, respectively, and  $A^{n-}$  is an n-valent intercalating anion (**Bini**  
44 **& Monteforte, 2018; Rives, 2001; Theiss et al., 2016**). Due to their interesting properties,  
45 including anion exchangeability, compositional flexibility, and biocompatibility, LDHs have  
46 found themselves various technological applications such as catalysis (**Yang et al., 2021**),  
47 sorption of pollutants (**Johnston et al., 2021**), bio-sensors (**Inmauddin, 2019**), drug storage  
48 delivery (**Bi et al., 2014**), photocatalytic water remediation (**L. Wang et al., 2021**) and  
49 antimicrobial materials (**León-Vallejo et al., 2019; Mishra et al., 2018**).

50 Nowadays, the fabrication of different materials avoiding biofouling or pathogenic bacteria  
51 proliferation is highly demanded (**Forano et al., 2018**). Under this aspect, LDH and LDH-  
52 composite materials were utilized in different antimicrobial applicational fields as water  
53 purification (**Kamal et al., 2018; Moaty et al., 2016**), food packaging (**Cheng et al., 2019;**  
54 **Tammaro et al., 2014**), wound healing dressings (**W. Zhang et al., 2020**), and textile industries  
55 (**Mallakpour et al., 2021**). In fact, many surveys showed that LDHs possess high potential as  
56 carriers of biological antibiotics due to their controlled release rate (**Djebbi et al., 2016;**  
57 **Salguero et al., 2020; Y. Wang & Zhang, 2012**). Antibiotics can treat bacterial infections,  
58 however, the excessive use of antibiotic had led to emergence of new antimicrobials and spread

59 of resistant strains (El-Shahawy et al., 2018; Williams et al., 2019), where the World Health  
60 Organization (WHO) estimates 700,000 global deaths per year due to antimicrobial resistance  
61 phenomenon (WHO, 2019). Moreover, some work had been reported on the loading of  
62 antimicrobial metallic nanoparticles as silver nanoparticles within the LDH matrix to achieve an  
63 efficient antimicrobial effect (Mao et al., 2019; Marcato et al., 2013; Mishra et al., 2013).  
64 Although these metallic nanoparticles-LDH composites exhibit significant antibacterial effects,  
65 the toxicity caused by their aggregation behavior is still the main challenge in such applications  
66 (Bélteky et al., 2019; Carja et al., 2009; Sharma & Prasher, 2020).

67 On the other hand, some recent studies proved that unmodified pristine LDHs could also act as  
68 efficient antibiotic-free antimicrobial materials where Moaty *et al.* showed that pristine  $Zn^{II}$ - $Fe^{III}$   
69 possess a durable antimicrobial activity against several Gram-positive and Gram-negative  
70 bacteria (Moaty et al., 2016). Peng *et al.* compared the antimicrobial properties of  $Mg^{II}$ -based  
71 LDHs to those of  $Zn^{II}$ -based ones and found out that the latter showed better antibacterial  
72 properties (Peng et al., 2018). Dutta *et al.* also reported contribution of  $Zn^{II}$ -based LDH to an  
73 efficient antimicrobial effect (Dutta et al., 2020). Moreover, Sanchez *et al.* found out that the  
74 antibacterial activity of pristine  $Zn^{II}$ - $Al^{III}$  LDHs could be enhanced by increasing the  $Zn^{II}$ - $Al^{III}$   
75 molar ratio (Lobo-Sánchez et al., 2018). Another recent study also highlighted the superior  
76 antimicrobial effect of  $Zn^{II}$ -based LDHs in comparison to  $Mg^{II}$  and  $Cu^{II}$ -based LDHs against  
77 *Corynebacterium ammoniagenes* (*C. ammoniagene*) (León-Vallejo et al., 2019). The  
78 antimicrobial properties of  $Cu^{II}$ -substituted  $Zn^{II}$ - $Al^{III}$  and its calcined products was also  
79 investigated, where calcined LDHs at higher temperatures performed better in antibacterial tests  
80 due to their smaller particle sizes promoting better contact (Mishra et al., 2018). Similarly,

81 Tabti *et al.* was able to prove the antimicrobial activity of ternary MgCuAl LDHs which was  
82 primary dependent on the Cu<sup>II</sup>:Al<sup>III</sup> molar ratio in the LDHs (Tabti *et al.*, 2020).

83 Despite the fact that several hypotheses were proposed by these studies to explain the  
84 antimicrobial effect of pristine LDHs or their derived metal oxides counterparts, the followed  
85 antibacterial mechanism is still not well understood. The partial dissolution of pristine LDHs into  
86 their constituents M<sup>II</sup> aqueous ions might constitute one possible mechanism explaining their  
87 antibacterial activities (León-Vallejo *et al.*, 2019; Li *et al.*, 2019; Peng *et al.*, 2018; Rocha  
88 Oliveira *et al.*, 2015). Although such antimicrobial mode of action was suggested, no real  
89 experimental set up was designed to validate it and no proof of experimental evidence was  
90 reported up until now. Moreover, these studies do not provide systematic approaches to  
91 determine in depth how to tune the physicochemical parameters of pristine LDHs to enhance the  
92 desired antimicrobial effect. Indeed, Vallejo *et al.* linked the antimicrobial effect of Zn<sup>II</sup> and Cu  
93 <sup>II</sup>-based LDHs to the amount of present cations in the powders, but not to the amount of released  
94 cations in the microbial culture media (León-Vallejo *et al.*, 2019). Rasouli *et al.* related the  
95 antibacterial efficiency of mixed metal oxide nanoparticles derived from Zn<sup>II</sup>-Al<sup>III</sup> LDHs directly  
96 to their crystallite sizes which may affect the solubility of the LDHs (Rasouli *et al.*, 2017). An  
97 insight concerning the role of released soluble zinc species on the toxicity of zinc oxide (ZnO)  
98 nanoparticles was established in some previous work (Esteban-Tejeda *et al.*, 2017; M. Li *et al.*,  
99 2011). However, these studies do not study systematically all parameters leading to the  
100 dissolution of the powders. Moreover, oxides have different compositional and physicochemical  
101 properties than those of LDHs, and would thus perform differently in terms of dissolution and  
102 antimicrobial effect. Under such circumstances, the study of the different parameters influencing  
103 the release behavior of Zn<sup>II</sup> in aqueous bacterial growth broth media, or any other metallic cation

104 possessing an antimicrobial activity out of LDHs is of utmost importance for evaluating and  
105 understanding such antimicrobial effect.

106 The exposure of LDH powders to aqueous solutions in which dissolution can certainly take place  
107 may play an important role in their several potential applications including antimicrobial ones.  
108 However, the subject had been unattended by the literature and the available evidences are  
109 scarce. In fact, some early studies evaluated the stability and dissolution properties of layered  
110 double hydroxides using thermodynamical approaches (**Allada, 2002; Allada et al., 2005;**  
111 **Bocclair & Braterman, 1999**). Such approaches are very interesting but also based on  
112 computational investigations, thus lacking real measurements in solution.

113 In 2011, Jobbagy *et al.* studied the release behavior of metallic ions from a synthetic chloride-  
114 containing MgAl LDH at a pH range of 1-9, in order evaluate its chemical stability and to assess  
115 the operating dissolution mechanisms (**Jobbágy & Regazzoni, 2011**). Another study mainly  
116 focused on the investigation of the cytotoxicity of hydrotalcites bearing different intercalated  
117 anions by evaluating the average dissolution profiles of Mg<sup>II</sup> from MgAl-CO<sub>3</sub> and MgAl-Cl in  
118 both simulated body and lysosomal fluids (**Baek et al., 2011**). The stability of these LDHs varied  
119 in accordance to the nature of intercalated anion, which also affected their cytotoxicity behavior.  
120 Moreover, Hibino reported the effect of crystallinity of carbonate-intercalated Mg-Al LDHs on  
121 their dissolution and anion exchange properties in different aqueous acetate buffer/NaCl media  
122 having a pH of 5.3 (**Hibino, 2014**). Compared to higher crystallinity LDHs, lower crystallinity  
123 ones showed lower extent of exchange of interlayer anions and greater weight loss of M<sup>II</sup>:Al<sup>III</sup>  
124 implying greater release of M<sup>II</sup> in aqueous media. Although these investigations considered real  
125 measurements of dissolved species in aqueous solutions, nonetheless, they were not devoted to

126 study in a systematic way the dissolution properties of LDHs. In other words, only the influence  
127 of one or a limited number of parameters on the dissolution properties of LDHs was considered,  
128 where in fact several factors may contribute to LDH dissolution, *e.g.*, crystallinity, nature of  
129 constituent metallic cations and  $M^{II}:Al^{III}$  molar ratio, as well as the type of intercalated anions  
130 within the layers. Thus, a global investigation concerning the different factors affecting the  
131 solubility of LDHs seems to be of great importance in this area.

132 In this context, the present study aims to evaluate systematically the influence of different  
133 parameters (crystallinity,  $M^{II}:Al^{III}$  ratio, type of intercalated anion  $\{CO_3^{2-}, Cl^-, NO_3^-, ClO_4^-\}$  and  
134 nature of  $M^{II}$  cations) on the solubility of pristine LDHs in broth media thus leading to their  
135 antimicrobial effect. The properties of Zn<sup>II</sup>-based LDHs had been systematically analyzed due to  
136 their relatively well-established antimicrobial behavior. The dissolution properties of the  
137 prepared  $M^{II}-Al^{III}$  LDHs were studied in growth media of *Escherichia coli* and *Staphylococcus*  
138 *aureus* bacteria, *i.e.* Lysogeny Broth (LB) and Tryptic Soy Broth (TSB), respectively. Finally,  
139 the relationship between the solubility and physicochemical properties of the LDHs and their  
140 antimicrobial activity was determined and subsequently the antimicrobial mechanism of pristine  
141 LDHs by metallic ions release was validated.

## 142 **2. Experimental section**

### 143 **2.1. Materials**

144 Aluminum nitrate nonahydrate ( $Al(NO_3)_3 \cdot 6H_2O$ ,  $\geq 99\%$ ), Zinc nitrate hexahydrate  
145 ( $Zn(NO_3)_2 \cdot 6H_2O$ ,  $\geq 99\%$ ), Copper (II) nitrate trihydrate ( $Cu(NO_3)_2 \cdot 3H_2O$ ,  $\geq 99\%$ ), Nickel (II)  
146 nitrate hexahydrate ( $Ni(NO_3)_2 \cdot 6H_2O$ ,  $\geq 99\%$ ), Cobalt (II) nitrate hexahydrate ( $Co(NO_3)_2 \cdot 6H_2O$ ,  
147  $\geq 98\%$ ), Magnesium nitrate hexahydrate ( $Mg(NO_3)_2 \cdot 6H_2O$ ,  $\geq 99\%$ ), Sodium carbonate ( $Na_2CO_3$ ),

148 Hydrochloric acid (HCl, 37%), Nitric acid (HNO<sub>3</sub>, 65%) Luria Bertani Broth (LB), Tryptic Soy  
149 Broth (TSB), Agar-agar, Phosphate Buffered Saline (PBS) tablets were purchased from SIGMA-  
150 ALDRICH. Ammonia solution (S.G. 0.88, 35%) was purchased from Fisher Scientific. Sodium  
151 hydroxide solution (1 mol.L<sup>-1</sup>) and Perchloric acid (HClO<sub>4</sub>, 60%) were purchased from VWR  
152 Chemicals and Raedel-de Haen, respectively. Antibiotic filter discs (6mm) and sterile cotton  
153 tipped swabs were purchased from Dominique Dutscher and COPAN, respectively. The  
154 standards used for ICP-MS tests were prepared in the laboratory from commercial solutions  
155 certified at 1000 ppm (Merck).

## 156 **2.2. Synthesis of carbonate intercalated layered double hydroxides**

157 Carbonate intercalated LDHs marked as M<sub>2</sub>Al:CO<sub>3</sub> (M= Zn, Cu, Ni, Co, Mg) and M<sub>3</sub>Al:CO<sub>3</sub>  
158 (M= Zn) were synthesized by a simple coprecipitation method (Kim et al., 2017). First a 0.05 M  
159 ammonium/ammonia solution was prepared then the pH of the solution was fixed to a value of  
160 10 using a solution of 1% HNO<sub>3</sub>. A solution of 0.4 mol.L<sup>-1</sup> M<sup>II</sup> and Al<sup>III</sup> nitrate salts having a  
161 molar ratio M<sup>II</sup>:Al<sup>III</sup> of 2:1 or 3:1 was added slowly using a peristaltic pump operated at 0.3  
162 mL.min<sup>-1</sup> into the ammonia solution. The pH was kept constant at 10 by an automatic titrator  
163 (736 GP Titrino, Metrohm) using a solution of 1 mol.L<sup>-1</sup> NaOH and 0.25 mol.L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>. For  
164 most of the synthesis, this was followed by a hydrothermal treatment in an autoclave at 80°C for  
165 24 hours to enhance the crystallinity, then the solids were washed for 4 times in deionized water  
166 and collected by centrifugation at 10000 rpm. Finally, the obtained LDH solids were dried in air  
167 at 80°C for 24 hours. Only one LDH, denoted as *Zn<sub>2</sub>Al:CO<sub>3</sub>.cop*, was not exposed to  
168 hydrothermal treatment, where the solid obtained directly after coprecipitation was washed and  
169 dried without any further treatment. The detailed experimental conditions for the synthesis of

170 carbonate intercalated LDHs are provided in **Table S1. (See supplementary material)** The  
171 synthesized LDHs were denoted as  $M_xAl:A^{n-}$ , where M is the constituent divalent metal of the  
172 LDH, Al corresponds to the constituent trivalent aluminum metal, x is the molar ratio of divalent  
173 to trivalent metal ( $M^{II}:Al^{III}$ ) and  $A^{n-}$  is the intercalated anion in the interlayer space.

### 174 **2.3. Synthesis of chloride, nitrate and perchlorate intercalated $Zn_2Al$ layered double** 175 **hydroxides**

176  $Zn_2Al:Cl$ ,  $Zn_2Al:NO_3$  and  $Zn_2Al:ClO_4$  LDHs were prepared by anion exchange reaction of  
177  $Zn_2Al:CO_3$ -hyd in presence of acid-alcohol mixed solutions following the method adapted by Iyi  
178 *et al.* (Iyi et al., 2011). Briefly, 1 mmol of  $Zn_2Al:CO_3$  was dispersed in 30 mL ethanol, then  
179 1.986 mmol of strong acid (HCl,  $HNO_3$ ,  $HClO_4$ ) present in 20 mL of ethanol solution was added,  
180 to avoid brutal contact between the solid and the acid thus limiting the dissolution of the LDHs.  
181 The whole exchange process was allowed to take place at  $50^\circ C$  for 1 hour under vigorous stirring  
182 and nitrogen ( $N_2$ ) flow to promote a total exchange. The samples were then filtered on a Buchner  
183 funnel, washed with distilled water and finally dried in air at  $80^\circ C$ .

### 184 **2.4.Characterization**

185  $M^{II}:Al^{III}$  molar ratios of LDHs were measured by Induced Couple Plasma Mass Spectroscopy  
186 (ICP-MS Agilent-7800). 10 mg of LDH were dissolved into 1%  $HNO_3$ . The calibration range  
187 was set as 10 to 3000 ppb. The detected isotopes were:  $^{27}Al$ ,  $^{66}Zn$ ,  $^{63}Cu$ ,  $^{60}Ni$ ,  $^{59}Co$ ,  $^{26}Mg$ .

188 Powder X-Ray Diffraction (PXRD) characterization at room temperature of the obtained  
189 powders was performed using a Panalytical X'Pert Pro MPD diffractometer in reflection  
190 geometry using Cu radiation ( $K\alpha_1 = 1.5406 \text{ \AA}$ ), a Ge(111) incident-beam monochromator, Soller  
191 slits of 0.02 rad, programmable divergence and anti-scatter slits. The irradiated area was fixed to

192 10mm x 10mm, and an X'Celerator detector was used. Data were collected from finely ground  
193 samples with a sample holder spinner and continuous rotation of sample to improve statistical  
194 representation of the samples. All the powder diffraction data were collected using the same  
195 procedure, *i.e.* at the same scattering angle range (between 5 and 70°) with a 0.0167°step and a  
196 speed of 1°/min. Lattice parameters were extracted from cell refinements of the LDH phases.  
197 The average crystallite size, calculated as XRD coherent domains, in the directions perpendicular  
198 ( $L_{(003)}$ ) and parallel ( $L_{(110)}$ ) to the hydroxide layers of the obtained LDHs were estimated from the  
199 full width at half maximum (FWHM) values of the respective diffraction reflections (003) and  
200 (110) using the Scherrer equation (Erreur ! Source du renvoi introuvable.):

$$201 \quad L = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

202 Where  $K$  is the Scherrer constant (0.9),  $\lambda$  is incident ray wavelength,  $\beta$  is the full width at half  
203 maximum in radians, and  $\theta$  is the Bragg angle (rad) (Cullity, 1978).

204 Raman spectroscopy characterizations were performed using a confocal Raman microscope  
205 (inVia® Qontor with a Peltier-cooled charge-coupled device (CCD) camera, Renishaw, Wotton-  
206 under-Edge, UK) equipped with a 532 nm laser, a 50x objective with 0.55 of numerical aperture,  
207 and a 2400 lines.mm<sup>-1</sup> grating. The spectral resolution was about 3 cm<sup>-1</sup> (10 acquisitions of 5  
208 seconds). Each sample was analyzed at different points to check the uniformity of the studied  
209 hydroxides. The laser power was changed in the range of 50 to 1 mW depending on the type of  
210 LDH sample and the spectra were recorded in the 400 to 4000 cm<sup>-1</sup> wavenumber range.

211 Infrared spectra were recorded in the mid-infrared range on a Bruker Vertex 70v spectrometer  
212 equipped with a KBr beam-splitter and a deuterated triglycine sulfate (DTGS) detector. An  
213 attenuated total reflection (ATR) accessory (Platinum ATR with a single reflection diamond

214 crystal, Bruker) was used for acquiring spectra of the LDH powders. The spectral resolution was  
215  $4\text{ cm}^{-1}$  and the accumulation time was 1 min (100 scans). Compartments containing the detector  
216 and the ATR accessory were under vacuum ( $<1\text{ hPa}$ ).

217 Specific surface area analysis of  $\text{Zn}^{\text{II}}\text{-Al}^{\text{II}}$  LDH samples were performed using the Brunauer-  
218 Emmet-Teller (BET) approach using a BELSORP MAX analyzer at 77K in  $\text{N}_2$ . The powders  
219 were degassed at  $120^\circ\text{C}$  prior to the measurements to remove any adsorbed water molecules.

220 Particle size distribution (PSD) of  $\text{Zn}^{\text{II}}\text{-Al}^{\text{II}}$  LDH samples was determined by Laser Diffraction  
221 using a Diffraction Mastersizer 3000 equipment from Malvern Instruments. 50 mg of dry powder  
222 was dispersed in 100 mL water using dispersion unit Hydro 2000 also from Malvern  
223 Instruments. Control tests were done in LB and TSB media for one LDH sample, and the PSD of  
224 this sample was the same as that done in distilled water, thus all other tests were conducted in  
225 distilled water for easier manipulation, time and economy saving. Three consecutive PSD  
226 measurements were carried out with a time interval separation of 2 minutes, in order to  
227 determine the volume median diameter ( $D_{v50}$ ) and the surface area mean diameter ( $D_{[3, 2]}$ ) for  
228 each sample.

229 The morphology of some LDH samples was examined by Scanning Electron Microscopy (SEM)  
230 performed on a JEOL JSM-IT500HR, with a Field Emission Gun (FEG) and a voltage of  
231 analysis situated in between 2kV and 5 kV. The analysis was performed under high vacuum, and  
232  $60\mu\text{m}$  diaphragm aperture was settled. Secondary Electron Detector (SED) was used for imaging.

### 233 **2.5.Partial dissolution experiments**

234 Partial dissolution profiles of the prepared LDHs into their constituents divalent and trivalent  
235 metal was measured by dispersing  $10\text{ mg.mL}^{-1}$  of LDHs in both Lysogeny Broth (LB) and

236 Tryptic Soy Broth (TSB). After a given incubation time of 48 hours at 37°C, the supernatants  
237 were collected by centrifugation. Finally, the metal concentration in the supernatant was  
238 measured by Induced Couple Plasma Mass Spectroscopy (ICP-MS Agilent-7800). Control  
239 partial dissolution experiments were also done in Milli-Q water under the same experimental  
240 conditions. Each experiment was repeated at least 3 times and the average dissolution of metals  
241 out of LDH powders was determined. The kinetic release profiles of Zn<sup>II</sup> from Zn<sub>2</sub>AlCO<sub>3</sub>-hyd  
242 and Zn<sub>2</sub>AlCO<sub>3</sub>-cop was studied in LB, TSB using the same mass concentration over 48 hours as  
243 well.

## 244 **2.6. Antibacterial experiments**

### 245 **2.6.1. Agar disc diffusion test**

246 The anti-bacterial activity of the synthesized LDHs was evaluated against two different  
247 pathogenic bacteria: *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) using agar disc  
248 diffusion assay. All experiments were repeated three times to ensure their reproducibility. First,  
249 *E. coli* and *S. aureus* were precultured overnight in 5 mL of LB and TSB respectively. LB and  
250 TSB agar plates were prepared, sterilized and solidified. Then, 250 µL of bacterial suspension  
251 were diluted to 1/1500 in sterile PBS and spread on LB and TSB agar plates using a sterile cotton  
252 swab to prepare bacterial lawns monolayers. Antibiotic filter discs of 6 mm were placed on these  
253 plates and 20 µL of LDH dispersions (10 mg.mL<sup>-1</sup>) were loaded on the respective discs. The  
254 plates were then incubated at 37°C for 48 h. Afterwards, bacterial inhibition zones were observed  
255 around the discs. The diameter of the circular zones was measured using a centimeter ruler and  
256 mean values of three repeated trials for each microorganism were recorded and represented in  
257 millimeters. A similar procedure was followed to investigate the antimicrobial activity of

258 metallic salts counterparts having a molar concentration of 3 mmol.L<sup>-1</sup> for Zn<sup>II</sup>, Ni<sup>II</sup>, Co<sup>II</sup> and  
259 Mg<sup>II</sup> salts (the average released concentration of metallic cations out of their LDH samples  
260 counterparts) and 10 mmol.L<sup>-1</sup> for Cu<sup>II</sup> salts (the average released concentration of Cu<sup>II</sup> out of  
261 Cu<sub>2</sub>Al:CO<sub>3</sub> LDH sample). Each test was repeated three times to ensure the reproducibility of our  
262 experiments and the mean inhibition diameter was calculated.

### 263 **2.6.2. Turbidimetric method for MIC determination**

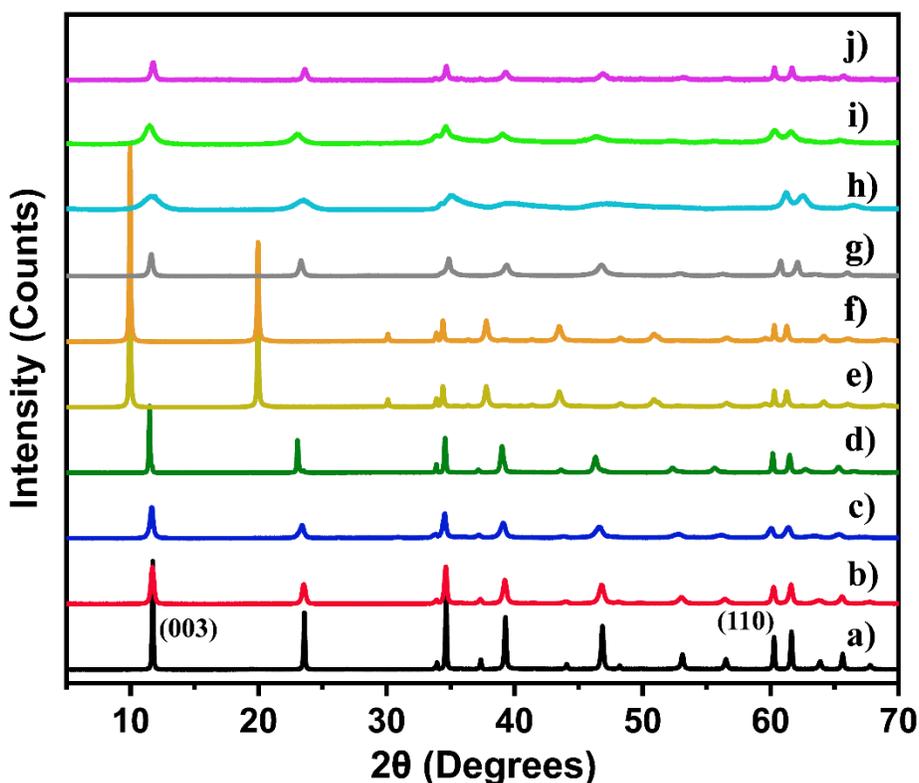
264 Broth microdilution turbidimetric method was used to determine the minimum inhibitory  
265 concentration (MIC) of the prepared Zn<sup>II</sup>-based LDH powders. *E. coli* and *S. aureus* were  
266 precultured in 5 mL of LB and TSB, respectively. An inoculum of each of strain was prepared  
267 and diluted to obtain an optical density of 0.1 at  $\lambda = 600$  nm (OD<sub>600</sub>=0.1). Zn<sup>II</sup>-based LDH  
268 powders were dispersed in broth culture media (LB or TSB), and two-fold dilution series were  
269 then prepared in a 96-well Micro-titer plate. Each well included 25  $\mu$ L of the diluted LDH  
270 powder and 175  $\mu$ L of bacterial inoculum. Un-inoculated broth wells containing the different  
271 used LDH dispersion concentrations were used as a negative standard growth control. As a  
272 positive control, 12 wells were prepared with 175  $\mu$ L of bacterial inoculum and 25  $\mu$ L of broth  
273 culture media. The Micro-titer plate was then sealed and incubated at 37°C under agitation for 48  
274 hours. The MIC value for each LDH sample was recognized as the lowest concentration that had  
275 no visible turbidity. Experiments were repeated at least 2 times for each sample and the wells  
276 corresponding to the obtained MIC values were the same.

## 277 **3. Results and discussion**

### 278 **3.1. Characterization of the LDHs**

279 **3.1.1. Structural features:  $M^{II}:Al^{III}$  molar ratio determination, Powder X-Ray**  
280 **Diffraction, Infra-Red and Raman spectroscopies analysis**

281 Carbonate-intercalated LDHs were synthesized by coprecipitation at a constant pH of 10 under  
282 vigorous stirring, and metallic salts solutions were prepared with  $M^{II}:Al^{III}$  molar ratios of 2 and 3  
283 (only for  $Zn^{II}-Al^{III}$  LDH).  $M^{II}:Al^{III}$  molar ratios of materials, measured by ICP-MS, were found  
284 to be very close to the expected values (**Table S1**). This implies that almost all the  $Al^{III}$  ions are  
285 co-precipitated with the  $M^{II}$  ions to obtain a solid with the stoichiometry of two or three  $M^{II}$   
286 atoms for each  $Al^{III}$  atom, thus corresponding potentially to ideal arrangements of the  $M^{II}$  and  
287  $Al^{III}$  cations in the brucite-like sheets (**Lieth, 1977**).



288  
289 **Figure 1.** PXRD patterns of the different prepared LDH samples: a)  $Zn_2Al:CO_3$ -hyd, b)  $Zn_2Al:CO_3$ -cop, c)  
290  $Zn_3Al:CO_3$ , d)  $Zn_2Al:Cl$ , e)  $Zn_2Al:NO_3$ , f)  $Zn_2Al:ClO_4$ , g)  $Mg_2Al:CO_3$ , h)  $Cu_2Al:CO_3$ , i)  $Ni_2Al:CO_3$ , j)  $Co_2Al:CO_3$ .

291 **Figure 1** shows the PXRD patterns of the prepared LDH samples having the characteristic  
 292 reflections of layered double hydroxides exhibiting a hexagonal lattice with rhombohedral 3R  
 293 symmetry (Cavani et al., 1991). The low angle peak situated in the  $2\theta$  range  $10^\circ$  and  $12^\circ$   
 294 depending on the type of LDH is indexed to the (003) basal reflection, whereas the peak located  
 295 at  $2\theta$  of approximately  $60^\circ$  is indexed to the (110) reflection. The unit cell lattice parameters  $a$   
 296 and  $c$  can be calculated from the position of these two peaks, with  $a = 2d_{(110)}$  and  $c = 3d_{(003)}$   
 297 (Mahjoubi et al., 2017).

298 The obtained parameters as well as the crystallite sizes  $L_{(003)}$  and  $L_{(110)}$ , calculated as XRD  
 299 coherent domains in directions perpendicular to the (003) and (110) planes respectively using  
 300 Scherrer's formula, are presented in **Table 1**. Slightly different lattice parameters of carbonate-  
 301 intercalated LDH samples may be due to the different atomic radii of their constituent metals and  
 302 their orientation within the LDH structure (M. Li et al., 2020). The values of the crystallite sizes  
 303  $L_{(003)}$  and  $L_{(110)}$  show that the prepared LDHs exhibit a relatively good crystallinity, with an only  
 304 exception for  $\text{Cu}_2\text{Al}:\text{CO}_3$  sample. This was also shown in the PXRD pattern of  $\text{Cu}_2\text{Al}:\text{CO}_3$   
 305 (**Figure 1-h**) which exhibited broader less intense diffraction peaks at (003) and (110) reflections  
 306 caused by poor long-range ordering (J. Li et al., 2017). Such decrease in crystallinity could be  
 307 actually attributed to the Jahn-Teller distortion effect of copper present in octahedral  
 308 coordination (Kannan et al., 2004).

309 **Table 1.** Unit cell parameters, interlamellar distance ( $d$ ),  $L_{(003)}$  and  $L_{(110)}$  of the prepared LDH samples. The data  
 310 was calculated by using the peak positions and the full width at half maximum (FWHM) of the (003) et (110)  
 311 diffraction peaks presented in Figure 1.

Sample	$a$ (Å)	$c$ (Å)	$d$ (Å)	$L_{(003)}$ * (nm)	$L_{(110)}$ * (nm)
$\text{Zn}_2\text{Al}:\text{CO}_3\text{-hyd}$	3.06	22.59	7.53	69	60
$\text{Zn}_2\text{Al}:\text{CO}_3\text{-cop}$	3.07	22.65	7.55	27	32

Zn <sub>2</sub> Al:Cl	3.07	23.1	7.7	46	32
Zn <sub>2</sub> Al:NO <sub>3</sub>	3.07	26.61	8.87	45	31
Zn <sub>2</sub> Al:ClO <sub>4</sub>	3.08	27.45	9.15	47	37
Zn <sub>3</sub> Al:CO <sub>3</sub>	3.08	22.86	7.62	25	35
Cu <sub>2</sub> Al:CO <sub>3</sub>	3.03	22.77	7.59	6	15
Ni <sub>2</sub> Al:CO <sub>3</sub>	3.05	22.59	7.59	13	23
Co <sub>2</sub> Al:CO <sub>3</sub>	3.05	22.56	7.52	23	37
Mg <sub>2</sub> Al:CO <sub>3</sub>	3.06	22.77	7.59	25	29

312 \* Crystallite sizes calculated as XRD coherent domains in directions perpendicular to (003) or (110) planes using Scherrer's  
313 formula. The cell parameters were calculated by using the peak positions of the (003) et (110) diffraction peaks presented in  
314 Figure 1.

315

316 The  $c$  parameter of Zn<sup>II</sup>-Al<sup>III</sup> carbonate-intercalated LDHs were found to be very close to that  
317 found in the literature (Crespo, 1997; J. Zhang et al., 2004). A slight difference could be noted  
318 between Zn<sub>2</sub>Al:CO<sub>3</sub>-hyd and Zn<sub>2</sub>Al:CO<sub>3</sub>-cop. A similar change in  $c$  lattice parameter was  
319 reported by Kovanda *et al.* (Kovanda et al., 2009) and Benito *et al.* (Benito et al., 2006) who  
320 assigned the decrease in the interlamellar space of the LDH after exposing it to hydrothermal  
321 treatment to the strengthening of the interaction between the interlayer anions and the hydroxide  
322 layers. As suggested by **Figure 1**, the application of hydrothermal treatment had also resulted in  
323 an increase of the integral intensity of (003) and (110) diffraction lines. This was associated with  
324 larger  $L_{(003)}$  and  $L_{(110)}$  crystallite sizes (**Table 1**), which also indicates an enhancement in the  
325 crystallinity of LDH phase in the treated sample and further ordering of the LDH crystal  
326 structure (Kovanda et al., 2009). Such effect of hydrothermal treatment on the crystallinity of  
327 the LDH samples is expected, where the application of hydrothermal treatment on hydrotalcites  
328 is mainly aimed to : (i) transform the small crystallites of the powders into larger more  
329 crystallized ones, (ii) transform amorphous precipitates into crystalline LDHs (Cavani et al.,

330 **1991**), or (iii) induce crystal rearrangement, thus decreasing the number of defects present in the  
331 crystals (**C.-C. Wang & Ying, 1999**). Miyata *et al.* had also reported a dramatic effect on the  
332 crystallite size induced by hydrothermal treatment of hydrotalcite compounds (**Miyata, 1980**).  
333 Actually, thermal treatment promotes the continuous dissolving of a significant part of the less  
334 thermodynamically stable particles obtained during the coprecipitation step, *i.e.* small particles  
335 with high surface energy and amorphous phases, while the resulting dissolved species  
336 recrystallize again adding to and forming more stable crystals (**Galvão et al., 2016**).

337 On the other hand, upon increasing the molar ratio of  $Zn^{II}:Al^{III}$  from 2 to 3, an increase in the  
338 lattice parameters  $a$  and  $c$  was observed (

339 **Table 2).** The first is related to the larger ionic radius of zinc compared to that of aluminum  
340 resulting in lattice expansion along the ab-plane, whereas the second is attributed to the reduction  
341 of layer electrostatic charge in the Zn<sub>3</sub>Al:CO<sub>3</sub> LDH leading to wider interlayer distance along the  
342 c-axis (**Lee et al., 2016**). Moreover, the crystallite sizes L<sub>(003)</sub> and L<sub>(110)</sub> had town down by 3 and  
343 2 times, respectively. In fact, it had been previously reported that an increase in M<sup>II</sup>:M<sup>III</sup> molar  
344 ratio is usually associated with a reduction in crystallinity degree due to a decrease in layer  
345 charges (**Ahmed et al., 2013**).

346 Moreover, upon exchanging carbonate anions in Zn<sub>2</sub>Al:CO<sub>3</sub> with other anions, the type of XRD  
347 patterns was unchanged, indicating that the product still kept the rhombohedral 3R symmetry (**Y.**  
348 **Liu & Yang, 2016**). However, the (003) peak was shifted to lower 2θ values compared with  
349 Zn<sub>2</sub>Al:CO<sub>3</sub> LDH, revealing an increase in the crystal layer spacing *c*, following the order:



351 The evolution of the *c* lattice parameter with the change in nature of intercalated anion is in  
352 agreement with those reported for hydrotalcite-like compounds (**Kloprogge et al., 2002;**  
353 **Bontchev et al., 2003**), thus indicating the success of the anion exchange reactions which was  
354 also ensured by Raman and IR spectroscopies. The interlamellar distance is actually related to  
355 the size, charge and orientation of the corresponding intercalated anions within the layers  
356 (**Mahjoubi et al., 2017**). The effect of anion exchange of CO<sub>3</sub><sup>2-</sup> with the other intercalating  
357 anions on the crystallinity of Zn<sub>2</sub>Al LDHs had been also investigated. The results shown in  
358 **Table 1** provide an evidence about the sensitivity of the crystallinity of LDHs toward anion  
359 exchange reactions, where the crystallite sizes L<sub>(003)</sub> and L<sub>(110)</sub>, had decreased by almost 1.5 times  
360 upon exchanging carbonate anions with chloride, nitrate and perchlorate. However, no

361 remarkable difference between the crystallite sizes of LDHs intercalated with  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  
362  $\text{ClO}_4^-$  was detected. We can thus assume that the exchange reactions of carbonate-intercalated  
363 LDHs, had led to a reduced crystallinity as a result of mechanical fragmentation of the  
364 crystallites rather than dissolution/recrystallization (**Salak et al., 2010**). In fact, anion exchange  
365 reactions in layered double hydroxides involves the breakage of the electrostatic interactions as  
366 well as the hydrogen bonds between the hydroxide layers and the outgoing anion and the  
367 reformation of these bonds with the incoming anions (**Radha et al., 2005**).

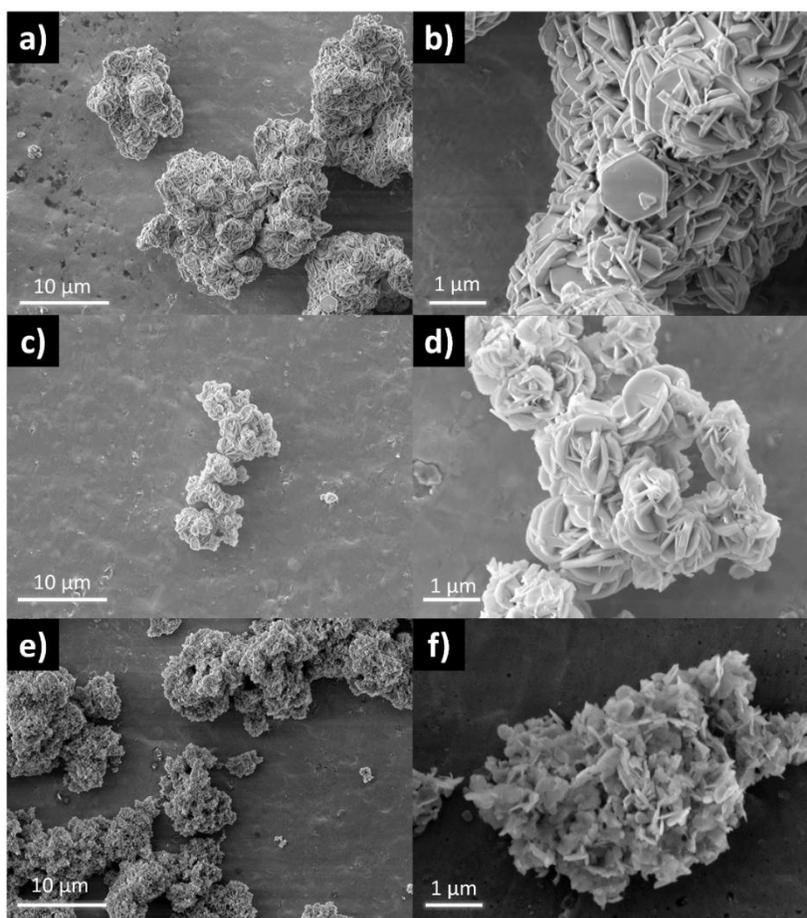
368 Finally, the purity of all the synthesized LDH powders was also checked out by Raman and IR  
369 spectroscopies, where no additional vibration bands assigned to amorphous phases have been  
370 detected (**Figures S1 and S2**).

### 371 **3.1.2. Textural features: Scanning Electron Microscopy imaging, BET specific surface** 372 **area and Particle Size Distribution**

373 As shown in **Figure 2**,  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-hyd}$  and  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-cop}$  as well as  $\text{Zn}_3\text{Al}:\text{CO}_3$  LDH samples  
374 adopt a “cauliflower-like” morphology, very similar to that reported by Scarpellini *et al.*  
375 (**Scarpellini et al., 2014**). The scaled samples exhibited distinct platelet like particles in a high  
376 degree of agglomeration, which is a well-known characteristic of LDHs (**Clark et al., 2019**).

377 In the absence of any hydrothermal treatment, the platelets hexagonal geometry of  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-}$   
378  $\text{hyd}$  was less sharp and defined (**Figure 2c,d**) than that of  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-hyd}$ . This highlights the  
379 lower crystallinity of the particles of  $\text{Zn}_2\text{Al}:\text{CO}_3$  unexposed to any hydrothermal treatment.  
380 However, the lateral particle size of hydrothermally treated  $\text{Zn}_2\text{Al}:\text{CO}_3$  ( $1050 \pm 130$  nm) was  
381 only slightly larger than that of the hydrothermally untreated sample ( $821 \pm 112$  nm). Thus, the  
382 larger crystallite size for  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-hyd}$  did not lead to significant changes on the particle size at

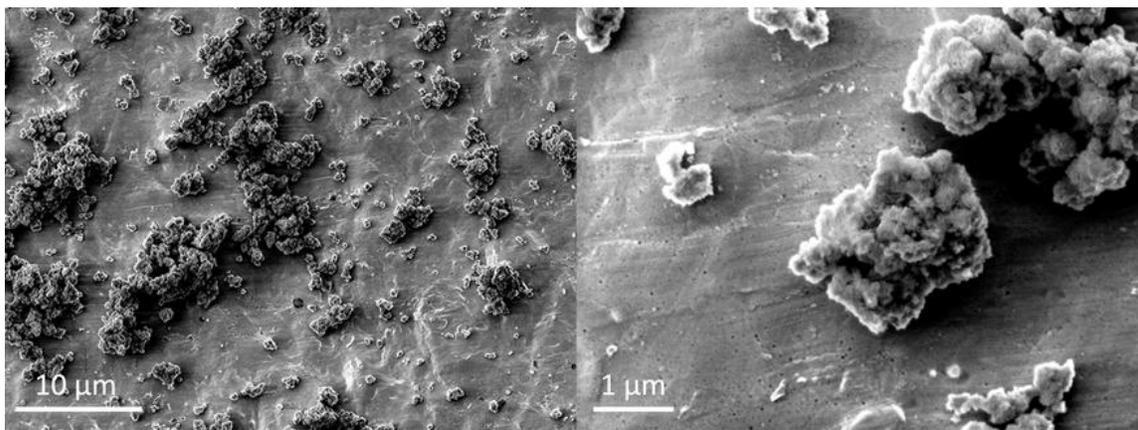
383 the micrometric range. In fact, it has been previously reported that the lateral size of LDHs  
384 prepared directly by coprecipitation without further hydrothermal treatment was almost the same  
385 as that of LDHs subsequently exposed to hydrothermal treatment, although their coherent  
386 crystallite domains were more than two times greater that of hydrothermally treated LDH  
387 (**Hibino, 2014**). On the other hand, Sun *et al.* reported a slight increase in the size of carbonate  
388 intercalated LDH nanoparticles upon exposing it to a hydrothermal treatment at 85°C for 7 hours  
389 (**Sun & Dey, 2015**). On the other hand, the SEM micrographs of Zn<sub>3</sub>Al:CO<sub>3</sub> hexagonal platelets  
390 having an average size lateral size 415 ± 45 nm, is much smaller and less defined than those of  
391 Zn<sub>2</sub>Al:CO<sub>3</sub>. In this case, the reduction in the average lateral size is in good agreement with the  
392 reduced coherent size of Zn<sub>3</sub>Al:CO<sub>3</sub> (**Table 1**).



393

394 **Figure 2.** SEM micrographs of: a,b)  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-hyd}$ , c,d)  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-cop}$ , e,f)  $\text{Zn}_3\text{Al}:\text{CO}_3$ .  
395 Moreover, upon exchanging carbonate anions in  $\text{Zn}_2\text{Al}:\text{CO}_3\text{-hyd}$  with chloride, nitrate and  
396 perchlorate anions a remarkable change in the morphology and aggregate size was observed  
397 **(Figure S3)**. The generation of defects in anion exchanged powders, especially for  $\text{Zn}_2\text{Al}:\text{NO}_3$   
398 and  $\text{Zn}_2\text{Al}:\text{ClO}_4$ , comes in line with the reduced crystallite sizes **(Table 1)**, and suggests again  
399 the occurrence of mechanical fragmentation of the crystallites in an ion exchange reaction rather  
400 than dissolution/recrystallization **(Salak et al., 2010)**.

401 **Figure 3** shows the morphology of  $\text{Cu}_2\text{Al}:\text{CO}_3$  exhibiting small globular distorted crystallites  
402 with uncertain length, similar to that of  $\text{CuMgAl}$  LDH obtained by Oliveira *et al.* **(Rocha**  
403 **Oliveira et al., 2015)**. In general, the synthesis of LDH containing  $\text{Cu}^{\text{II}}$  as the only divalent  
404 metal is somehow difficult due to the Jahn teller distortion phenomena **(H. Liu et al., 2010)**  
405 which may had led to a distortion and presence of defects in its structure, as suggested by its  
406 PXRD pattern.



407  
408 **Figure 3.** SEM micrographs of  $\text{Cu}_2\text{Al}:\text{CO}_3$  LDH.

409 The dissolution behavior of LDHs is considered as a surface-controlled reaction which is  
410 expected to be highly determined by their particle size **(Rojas, 2016)**. For this reason, the

411 specific surface area and particle size distribution of the synthesized different  $Zn^{II}$ - $Al^{III}$  LDHs  
412 were studied by BET approach and Laser Diffraction, respectively.

413 BET provides a suitable method to measure specific surface area of LDH powders which is  
414 directly linked to their sizes. Moreover, particle size distribution (PSD) also gives information  
415 about the LDH particle sizes, which usually are aggregates of crystallites (**Herrero et al., 2008**).

416 The  $D_{v50}$  is the volume median diameter which divides the population exactly into two equal  
417 halves, whereas the  $D_{[3, 2]}$  is the surface area mean diameter, which is more relevant when  
418 specific surface area is important, e.g. bioavailability, reactivity and dissolution (**Rawle, 2003**).

419 The specific surface areas of  $Zn^{II}$ -based LDHs determined by BET ( $S_{BET}$ ),  $D_{v50}$  and  $D_{[3, 2]}$  values  
420 are reported in

421 **Table 2.**

422

423 **Table 2.** Specific surface area determined by BET ,  $D_{v50}$  and  $D_{[3, 2]}$  determined by PSD for the different prepared  
 424  $Zn^{II}$ - $Al^{III}$  LDH samples. The PSD curves are presented in Figure 4.

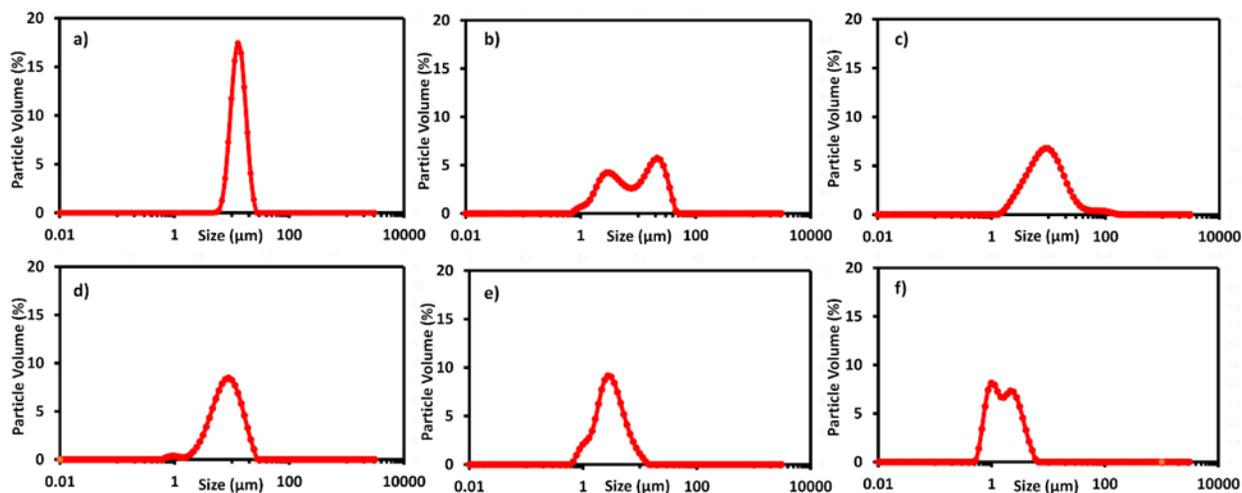
Sample	$S_{BET}$ ( $m^2 \cdot g^{-1}$ )	$D_{v50}$ ( $\mu m$ )	$D_{[3, 2]}$ ( $\mu m$ )
$Zn_2Al:CO_3$ -hyd	6.2	$13.7 \pm 0.4$	$13.2 \pm 0.3$
$Zn_2Al:CO_3$ -cop	19	$9.6 \pm 0.3$	$5.2 \pm 0.2$
$Zn_3Al:CO_3$	13.3	$9.4 \pm 0.3$	$7.3 \pm 0.2$
$Zn_2Al:Cl$	16.7	$8.4 \pm 0.1$	$6.4 \pm 0.1$
$Zn_2Al:NO_3$	22.5	$3.1 \pm 0.1$	$2.6 \pm 0.1$
$Zn_2Al:ClO_4$	24.8	$1.7 \pm 0.1$	$1.5 \pm 0.1$

425 Compared to  $Zn_2Al:CO_3$ -hyd LDH sample, which showed a monomodal narrow size distribution  
 426 of particle aggregates having a median diameter of  $13.7 \mu m$  (**Figure 4a**),  $Zn_2Al:CO_3$ -cop showed  
 427 a bimodal size distribution composed of both individual particles and aggregates with broader  
 428 size distribution having an average median size of  $9.6 \mu m$  (**Figure 4b**). The larger size and  
 429 narrow particle distribution of  $Zn_2Al:CO_3$ -hyd sample could be explained by the aging in  
 430 hydrothermal conditions which leads to recrystallization and Ostwald ripening processes (**Rojas,**  
 431 **2016**). Additionally, the larger  $S_{BET}$  and smaller  $D_{[3, 2]}$  for  $Zn_2Al:CO_3$ -cop supported such  
 432 results. The reduction of the surface area of LDHs subsequently exposed to a hydrothermal  
 433 treatment could be attributed to the recrystallization of small crystallites and amorphous particles  
 434 and subsequent increase of the LDH crystallite size (**Kovanda et al., 2009**), which is also  
 435 confirmed by our XRD and SEM results presented in **Figure 1** and **Figure 2**, respectively.

436 On the other hand, upon increasing the molar ratio of  $Zn^{II}:Al^{III}$  from 2 to 3, the LDH powder  
 437 showed a broader particle size distribution.  $Zn_3Al:CO_3$  exhibited a monomodal distribution  
 438 similar to that of  $Zn_2Al:CO_3$ , yet with a short arm located at around  $100 \mu m$  and a smaller  
 439 aggregated particles fraction ( $\sim 27\%$ ) in the range of  $1.5-5 \mu m$  (**Figure 4c**), which was absent in  
 440 the case of  $Zn_2Al:CO_3$ -hyd.

441 Similarly, upon exchanging carbonate anions in  $\text{Zn}_2\text{Al}:\text{CO}_3$  with other intercalating anions, *i.e.*  
442  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{ClO}_4^-$ , a significant fraction of smaller agglomerates and/or individual particles  
443 appeared (**Figure 4d,e,f**). Such decrease in agglomeration degree imposed by anionic exchange  
444 reactions could also be seen through the SEM micrographs presented in **Figure S3**. This was  
445 also associated with a remarkable increase in  $S_{\text{BET}}$  and smaller  $D_{\text{V50}}$  and  $D_{[3, 2]}$  sizes, especially for  
446 the nitrate and perchlorate intercalated samples (

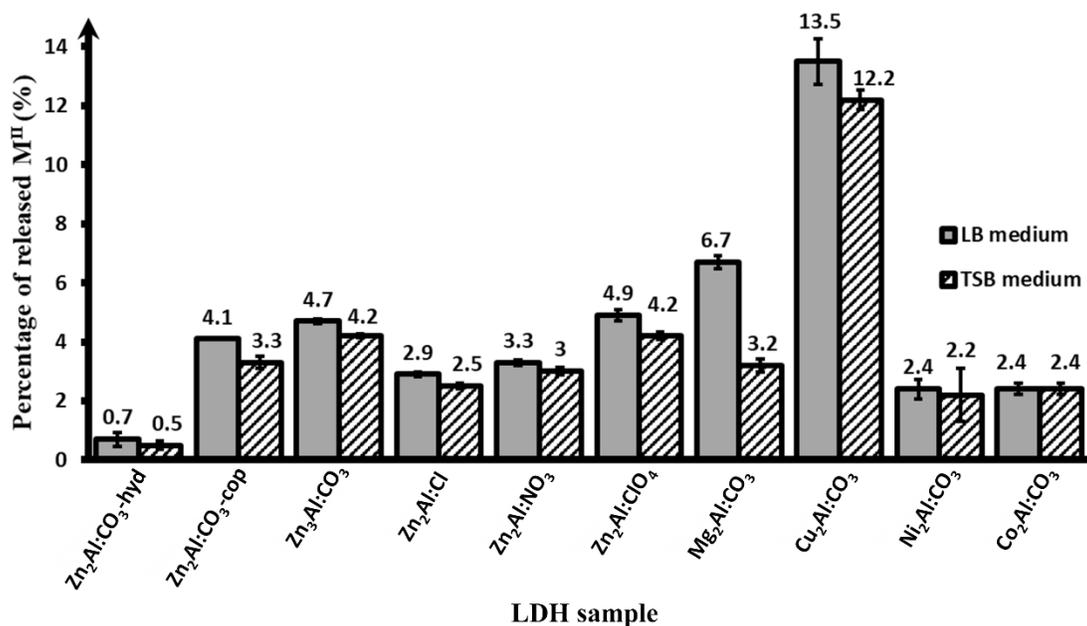
447 **Table 2).**



448  
449 **Figure 4.** Particle Size Distribution (PSD) curves in water dispersion of: a)  $Zn_2Al:CO_3$ -hyd, b)  $Zn_2Al:CO_3$ -cop, c)  
450  $Zn_3Al:CO_3$ , d)  $Zn_2Al:Cl$ , e)  $Zn_2Al:NO_3$ , and f)  $Zn_2Al:ClO_4$ .

### 451 **3.2.Metal partial dissolution experiments**

452 The partial dissolution of all the synthesized LDH powders into  $Al^{III}$  and their constituent  
453 divalent metals  $M^{II}$  in LB and TSB liquid broth media, of pHs 6.7 and 7 respectively, was  
454 studied after 48 h of incubation at  $37^\circ C$ . The negative blanc tests of pure LB and TSB media  
455 ensured that the media where the study had taken place did not contain any amounts of  $Al^{III}$  or  
456 any other divalent metal included in the study, *e.g.*,  $Zn^{II}$ ,  $Cu^{II}$ ,  $Ni^{II}$ ,  $Co^{II}$ ,  $Mg^{II}$ , prior to the  
457 addition of LDH powders. The partial dissolution profiles of all synthesized LDH samples are  
458 revealed in **Figure 5**.



459

460 **Figure 5.** Partial dissolution profiles of all synthesized LDH samples into M<sup>II</sup> in LB and TSB media in terms of  
 461 average percentage compared to the initial amount present in the LDH.

462 In this study, the dissolution of LDHs into their constituent divalent metals was uniquely  
 463 considered, since the dissolution of Al<sup>III</sup> in both LB and TSB media were below the detection  
 464 point for all the tested samples. In fact, Peng *et al.* had previously shown that only divalent  
 465 cations were released from LDH powders into a certain biological medium ( $\alpha$ -MEM), whereas  
 466 soluble trivalent cations (Al<sup>III</sup> and Fe<sup>III</sup>) were not released (Peng *et al.*, 2018). Jobbagy *et al.* also  
 467 reported that the dissolution of Al<sup>III</sup> out of Mg<sub>2</sub>Al:Cl LDH was below the limit of detection in the  
 468 range  $5 \leq \text{pH} \leq 9.0$ , and only leaching of Mg<sup>II</sup> was observed (Jobbágy & Regazzoni, 2011).

469 Control partial dissolution experiments of the synthesized LDH powders were also done in Milli-  
 470 Q distilled water having a pH of 6.98 very close to that of LB and TSB, where the release of both  
 471 divalent and trivalent metals was below the detection point for all the LDH samples (Table S2).  
 472 Such behavior could provide an evidence that the chemical species contained in LB and TSB  
 473 broth are able to increase the solubility of LDHs by the formation of complex species with their

474 constituent divalent metal. In fact, several studies have previously shown that the solubility and  
475 metal release from zinc oxide (ZnO) powders was greatly enhanced in biological complex media  
476 as LB, TSB and MH (Mueller Hinton) broths, which may be due to the ability of zinc present in  
477 the powder to react with amino acids and proteins to form complex organometallic compounds  
478 (Esteban-Tejeda et al., 2017; S. Liu et al., 2014; Pasquet et al., 2014). In the present case,  
479 proteins and amino acids present in LB and TSB liquid broths were probably the chemical  
480 species that might formed complexes with  $M^{II}$  ions and provided a superior dissolution of the  
481 powders compared to that in water.

482 Furthermore, in order to ensure that thermodynamic equilibrium was reached within 48 hours of  
483 LDH dispersion incubation, the evolution of  $Zn^{II}$  released from  $Zn_2Al:CO_3$ -hyd and  $Zn_2Al:CO_3$ -  
484 cop was monitored over time. As suggested by **Figure S4**, thermodynamic equilibrium was  
485 reached for  $Zn_2Al:CO_3$ -hyd and  $Zn_2Al:CO_3$ -cop within 8 and 6 hours, respectively. The faster  
486 release profile in  $Zn_2Al:CO_3$ -cop was probably due to the presence of a significant fraction of  
487 smaller particles/agglomerates (**Figure 4**) which had provided a larger exposed surface area in  
488 contact with the dissolution media, thus promoting greater release of  $Zn^{II}$  in aqueous broth  
489 solutions (Rojas, 2016).

490 Thus, the following subsections mainly focus on the effect of different parameters, *e.g.*  
491 crystallinity,  $M^{II}:Al^{III}$  molar ratio, type of intercalated anion and nature of divalent metal and  
492  $M^{II}:Al^{III}$  on the partial dissolution of different LDHs into their corresponding constituent divalent  
493 metals in LB and TSB aqueous dispersions.

#### 494 **3.2.1. Effect of crystallinity in $Zn_2Al:CO_3$**

495 The application of a post-synthetic hydrothermal treatment is an important step evaluated in this  
496 study for obtaining  $Zn_2AlCO_3$  LDH samples having different crystallinities:  $Zn_2Al:CO_3$ -hyd and  
497  $Zn_2Al:CO_3$ -cop. Both samples were prepared using the same experimental procedures, where the  
498 only difference was the application of a hydrothermal treatment for  $Zn_2Al:CO_3$ -hyd, while  
499  $Zn_2Al:CO_3$ -cop was not exposed to any post-synthetic treatment procedure. Subsequently, the  
500 two LDHs possessed different structural and textural properties, where  $Zn_2Al:CO_3$ -cop exhibited  
501 lower crystallinity revealed by their smaller crystallite sizes (**Table 1**), larger surface area,  
502 smaller median size and surface area mean of agglomerates (

503 **Table 2)** than those of the treated one.

504 The crystallinity effect on the stability and dissolution properties of LDHs was studied by  
505 evaluating the amount of released  $Zn^{II}$  from hydrothermally treated and untreated  $Zn_2Al:CO_3$   
506 LDH samples in LB and TSB media. It could be noticed from **Figure 5**, that the dissolution of  
507  $Zn^{II}$  in both samples was slightly higher in LB medium than TSB. This might be simply  
508 attributed to the difference in nature and composition of the biological growth media, in addition  
509 to the slightly lower pH of LB (6.63) compared to that of TSB (6.97), where it is known that the  
510 solubility of LDHs is highly pH-dependent and their dissolution accelerates with increasing  
511 acidity (**Zheng et al., 2013**).

512 In both media, the amount of the leached  $Zn^{II}$  out of  $Zn_2Al:CO_3$ -cop was enhanced by almost 6  
513 times in comparison to that of  $Zn_2Al:CO_3$ -hyd. Such result is consistent with that of Hibino, who  
514 found out that the release of  $Mg^{II}$  out of lower crystallinity  $Mg^{II}-Al^{III}$  LDH directly obtained after  
515 co-precipitation was higher than that of the same LDH subsequently exposed to hydrothermal  
516 treatment (**Hibino, 2014**). Therefore, the lower crystallinity of  $Zn^{II}-Al^{III}$  LDH as well as their  
517 smaller aggregated sizes and larger surface area promotes the release of more  $Zn^{II}$  ions in broth  
518 culture media.

### 519 **3.2.2. Effect of $M^{II}:Al^{III}$ molar ratio in $Zn_2Al:CO_3$ LDHs**

520 The impact of  $M^{II}:M^{III}$  molar ratio on divalent metal dissolution capacity of LDHs was another  
521 important parameter investigated in our study, by which as far as our knowledge such impact  
522 have not been studied before. Such influence was examined by comparing the partial dissolution  
523 profiles of  $Zn^{II}$  of the two prepared LDH samples:  $Zn_2Al:CO_3$ -hyd and  $Zn_3Al:CO_3$  having a

524  $Zn^{II}:Al^{III}$  molar ratio of 2 and 3 respectively. The two samples were prepared using a similar  
525 coprecipitation experimental procedure followed by a hydrothermal treatment at 80°C.

526 As shown in **Figure 5**, the partial dissolution profiles of  $Zn^{II}$  increases significantly by about 7  
527 and 8 times in LB and TSB media respectively, when the molar ratio of  $Zn^{II}:Al^{III}$  was increased  
528 from 2 to 3.

529 It is worth mentioning here that the crystallinity of these samples and the particles/agglomerates  
530 sizes as well their surface areas were not the same for these two samples. The crystallite sizes  
531 determined by XRD (**Table 1**Erreur ! Source du renvoi introuvable.) were reduced by at least  
532 two times upon increasing the  $Zn^{II}:Al^{III}$  molar ratio. It was actually noticed in the previous section  
533 that the crystallinity of LDHs could play an important role on their dissolution behavior, where  
534 the solubility of LDHs in aqueous solutions was expected to be enhanced as their crystallinity  
535 decreases (**Section 3.2.1**). Thus, the higher dissolution behavior presented in  $Zn_3Al:CO_3$  could be  
536 either assigned to its higher zinc content initially present in the structure, or to its lower  
537 crystallinity, or to the combined effect of these two factors.

### 538 **3.2.3. Effect of intercalated anion**

539 The influence of intercalating four different interlayer anions:  $CO_3^{2-}$ ,  $Cl^-$ ,  $NO_3^-$  and  $ClO_4^-$  in  
540  $Zn_2Al$  LDH layers, on their partial dissolution into  $Zn^{II}$  ions was investigated. The partial  
541 dissolution profiles of these LDHs (**Figure 5**), prove that changes in the nature and composition  
542 of the anion in the hydrotalcite interlayer region exhibited an important effect on the solubility of  
543  $Zn^{II}-Al^{III}$  LDHs. For instance, the release of  $Zn^{II}$  increases by about 4 times in LB medium upon  
544 exchanging carbonate anions with chloride and nitrate ones, and 6 times in case of perchlorate  
545 compared to carbonate intercalated sample within the same LB medium. Another important point

546 to note here is that the difference in the dissolution behavior in LB and TSB media, having  
547 slightly different pHs, is more pronounced in case of carbonate intercalated Zn<sub>2</sub>Al LDH than  
548 other samples having other intercalated anions. This could be explained by the urge to protonate  
549 both hydroxyl groups and carbonate anions in order for the dissolution to occur in carbonate  
550 intercalated LDH (**Hibino, 2014**), thus making Zn<sub>2</sub>Al:CO<sub>3</sub> LDH sample more sensitive to the pH  
551 of the aqueous media.

552 It is also remarkable that the increasing solubility of LDHs here followed the same trend as that  
553 of the value of c parameter obtained by XRD (**Section** Erreur ! Source du renvoi introuvable.),  
554 and the opposite trend to that of the order of affinity of LDHs toward intercalating anions  
555 previously described in the literature [**27,28**]:



557 Thus, one could conclude that the lower the affinity of the intercalated anion toward the Zn<sub>2</sub>Al  
558 LDH structure, the lower the stability of the LDH in broth media.

559 In fact, some previous studies showed that the dissolution of LDHs was strongly dependent on  
560 the type of intercalated anion, where Baek *et al.* reported that chloride intercalated MgAl-LDH  
561 was less stable and released more magnesium ions than carbonate intercalated MgAl-LDH in  
562 simulated body conditions (**Baek et al., 2011**). Other theoretical thermodynamic study had also  
563 shown that NO<sub>3</sub><sup>-</sup> intercalated hydrotalcites were more soluble than CO<sub>3</sub><sup>2-</sup> intercalated ones and  
564 even small amounts of carbonate could change the solubility of a nitrate phase by several orders  
565 of magnitude (**R. k. Allada, 2002**).

566 However, one important parameter neglected by these studies (**Allada, 2002; Baek et al., 2011**),  
567 is the difference in crystallinity and the structure of the LDHs having different intercalated

568 anions. As previously mentioned in **Section 3.1.1**, exchanging carbonate anions in  $Zn_2Al$  LDHs  
569 with other anions less favored by the LDH structure promoted a remarkable decrease in the  
570 crystallinity of the latter associated with a decrease in aggregate sizes (

571 **Table 2)** and generation of defects in the structure (**Figure S3**).

572 Therefore, the poor crystallinity and presence of defects in Zn<sub>2</sub>Al LDHs is a first explanation for  
573 the higher dissolution of LDHs obtained by anion exchange. The second one is the nature of  
574 intercalated anion itself, which may weaken the binding between the metal cations and the  
575 layers, thus promoting higher dissolution behavior.

#### 576 **3.2.4. Effect of divalent metal**

577 In order to examine the relationship between the constituent divalent metal M<sup>II</sup> of the LDHs and  
578 their dissolution properties, different LDHs having a molar ratio of M<sup>II</sup>:Al<sup>III</sup>=2 were synthesized  
579 coprecipitation method followed by a hydrothermal treatment at 80°C for 24 hours. The  
580 intercalated anion in all these LDH samples was CO<sub>3</sub><sup>2-</sup>, and the divalent metal was changed  
581 accordingly: M<sup>II</sup>= Zn<sup>II</sup>, Mg<sup>II</sup> Cu<sup>II</sup>, Ni<sup>II</sup>, or Co<sup>II</sup>.

582 According to **Figure 5**, the partial dissolution profile of M<sub>2</sub>Al:CO<sub>3</sub> LDHs into M<sup>II</sup> in both LB  
583 and TSB media followed the following trend:



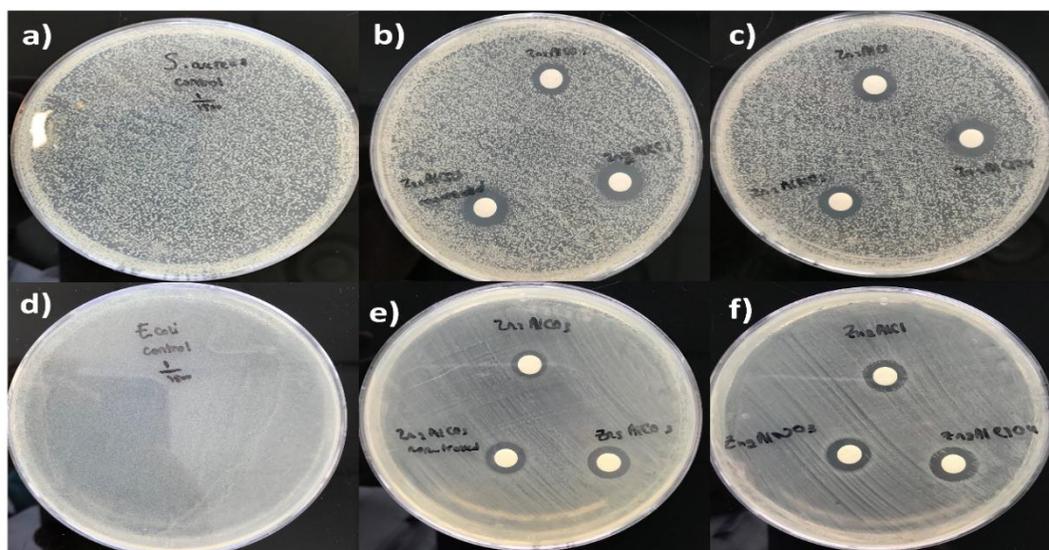
585 **Table S3** provides the relative pK<sub>sp</sub> values of the different divalent metal hydroxides  
586 counterparts of the different synthesized LDH samples. In fact, Boclair *et al.* had previously  
587 related the relative stabilities of LDHs to their corresponding metal hydroxides (**Boclair &**  
588 **Braterman, 1999**). That is for a given M<sup>II</sup> cation, the higher the solubility of M<sup>II</sup>(OH)<sub>2</sub>, the less  
589 stable its corresponding LDH is. Thus, we tried to correlate the solubility of M<sup>II</sup>(OH)<sub>2</sub> to the  
590 partial dissolution profiles of divalent metals from M<sub>2</sub>Al:CO<sub>3</sub> in LB and TSB media. Our results  
591 agreed well with the proposed hypothesis by Boclair *et al.*, by which lower dissolution is

592 observed with increasing  $M^{II}(\text{OH})_2$   $pK_{sp}$  values. The dissolution driving force is dictated by  
593 thermodynamics, and the solubility of LDHs varies according to the nature of the constituent  
594 metal ions. One sole exception was noted in the case of  $\text{Cu}_2\text{Al}:\text{CO}_3$  LDH which seemed to be  
595 highly unstable thus generating an amplified release of  $\text{Cu}^{II}$  although  $\text{Cu}(\text{OH})_2$  exhibited lower  
596 solubility compared to those of the other studied divalent metals. This surprising result, may be  
597 attributed to the poor crystallinity of  $\text{Cu}_2\text{Al}:\text{CO}_3$  (**Figure 1.h**), very small crystallite sizes ( $L_{(003)} \approx$   
598  $6\text{nm}$  and  $L_{(110)} \approx 15\text{ nm}$ ), and its distorted very irregular structure revealed by its SEM  
599 micrographs (**Figure 3**), thus indicating the presence of defects due to Jahn Teller distortion  
600 phenomena.

### 601 **3.3.Antibacterial activity**

#### 602 **3.3.1. Influence of nature of divalent metal on antibacterial activity**

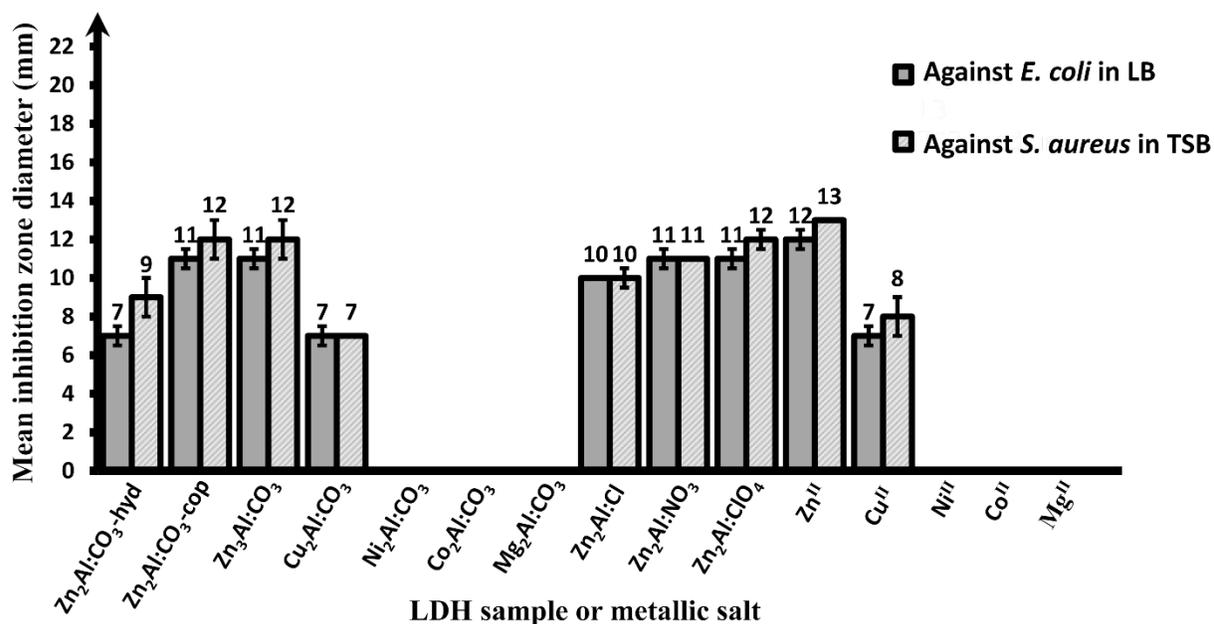
603 The antibacterial activities of the different prepared LDH samples were conducted against both  
604 Gram-positive and Gram-negative bacterial strains, *S. aureus* and *E. coli* respectively, using the  
605 agar disk diffusion method. The antimicrobial activity tests were also done using metallic salts  
606 solutions as references. The generated inhibition zones of some LDH samples against both tested  
607 bacteria are displayed in **Figure 6**.



608

609 **Figure 6.** Antibacterial agar disk diffusion tests against: a) *S. aureus* control, b) *S. aureus* using  $Zn_2Al:CO_3$ -hyd  
 610 (top),  $Zn_2Al:CO_3$ -cop (bottom left) and  $Zn_3Al:CO_3$  (bottom right), c) *S. aureus* using  $Zn_2Al:Cl$  (top),  $Zn_2Al:NO_3$   
 611 (bottom left) and  $Zn_2Al:ClO_4$  (bottom right), d) *E. coli* control, e) *E. coli* using  $Zn_2Al:CO_3$ -hyd (top),  $Zn_2Al:CO_3$ -  
 612 cop (bottom left) and  $Zn_3Al:CO_3$  (bottom right), f) *E. coli* using  $Zn_2Al:Cl$  (top),  $Zn_2Al:NO_3$  (bottom left) and  
 613  $Zn_2Al:ClO_4$  (bottom right).

614 As shown in **Figure 7**, only  $Zn^{II}$  and  $Cu^{II}$ -based LDHs were able to impose an antimicrobial  
 615 effect against *E. coli* and *S. aureus*.  $Zn^{II}$ -based LDHs provided inhibition zone diameters ranging  
 616 from 7 to 13 mm depending on the type of LDH and the amount of zinc ions released. These  
 617 obtained values were similar to those found for pristine LDHs prepared Li et al. (M. Li et al.,  
 618 **2020**) and Mishra et al. (Mishra et al., 2018). However, other antimicrobial pristine  $Zn^{II}$ -based  
 619 LDHs possessed greater bacterial inhibition zones (Dutta et al., 2020; Moaty et al., 2016). Such  
 620 difference in the antimicrobial performance may be related to the properties of the tested LDHs,  
 621 the different tested bacterial strains, the applied LDH dosage as well as to the different way the  
 622 antimicrobial agar diffusion test assay was carried out.



623  
 624 **Figure 7.** Antimicrobial activity of LDH samples and metallic ion solutions against *S. aureus* and *E. coli* in terms of  
 625 mean zone of inhibition.

626 In fact, previous studies had established the capability of pristine Zn<sup>II</sup>-containing LDH powders  
 627 to act as efficient antimicrobial materials (Moaty et al., 2016; Peng et al., 2018). Another recent  
 628 study had also demonstrated that modified as well as unmodified Cu<sup>II</sup>-based hydrotalcites could  
 629 exhibit a bactericidal effect that is expected to be dependent on the amount of released copper  
 630 ions (Rocha Oliveira et al., 2015).

631 Therefore, the antibacterial activity of pristine LDHs is primarily dependent on the nature of  
 632 constituent divalent metals where Zn<sup>II</sup>-based LDHs performed best in the agar disk diffusion test  
 633 assay followed by Cu<sup>II</sup>-based LDH sample.

### 634 3.3.2. Difference in antibacterial activity between Zn<sup>II</sup> and Cu<sup>II</sup>-based LDHs

635 The fact that Zn<sup>II</sup>-containing LDHs were able to establish a superior antibacterial activity to  
 636 those of other LDHs, even at low Zn<sup>II</sup> release profile as is the case for Zn<sub>2</sub>AlCO<sub>3</sub>-hyd, could be  
 637 attributed to the nature of the divalent metal present in the LDH itself. Where the tested Zn<sup>II</sup> salts

638 also showed an enhanced antibacterial activity in comparison with the  $\text{Cu}^{\text{II}}$  salts (**Figure 7**). This  
639 highlights again the specificity of the antimicrobial effect toward the nature of the divalent metal.  
640 Indeed, both  $\text{Zn}^{\text{II}}$  and  $\text{Cu}^{\text{II}}$  metallic ions possess similar antibacterial mechanisms by which they  
641 can cause damages to membrane functions by blocking the transport of nutrients and substances  
642 in and out the membrane, or bind to bacterial intracellular DNA leading to their denaturation  
643 (**Borkow & Gabbay, 2005; Mittapally et al., 2018; Peng et al., 2018**). However, the difference  
644 in the antimicrobial efficiency between different divalent metals, in this case  $\text{Zn}^{\text{II}}$  and  $\text{Cu}^{\text{II}}$ , is  
645 actually dependent on several factors, including the role each metal plays in normal metabolism,  
646 and the presence of specific binding sites for each metal (**Borkow & Gabbay, 2005**). Therefore,  
647 microorganisms may be more sensitive to one metal than the other (**Bruins et al., 2000**), *i.e.* in  
648 this case *E. coli* and *S. aureus* were more sensitive to  $\text{Zn}^{\text{II}}$  than  $\text{Cu}^{\text{II}}$ .

### 649 **3.3.3. Influence of the solubility of LDHs on its antibacterial activity**

650 Based on the proposed antibacterial mechanisms of LDHs provided in **Table 3**, the antimicrobial  
651 activity of LDHs may be due to: (i) electrostatic surface interactions with the negatively charged  
652 bacterial membranes, (ii) generation of biological toxic reactive oxygen reactive species (ROS),  
653 (iii) release of antimicrobial metallic ions. Taking the third mechanism specifically into  
654 consideration, the inability of  $\text{Ni}^{\text{II}}$  and  $\text{Co}^{\text{II}}$ -based LDHs to demonstrate an antibacterial activity  
655 could be explained by the lower release profiles of  $\text{Ni}^{\text{II}}$  and  $\text{Co}^{\text{II}}$  out of  $\text{Ni}_2\text{AlCO}_3$  and  $\text{Co}_2\text{AlCO}_3$   
656 LDHs respectively. Their respective metallic salts were also not able to impose the desired  
657 antimicrobial effect at a concentration similar to that of the released ones. Whereas in case of  
658  $\text{Cu}_2\text{AlCO}_3$ , the release profile of  $\text{Cu}^{\text{II}}$  was remarkably high (in the range of  $10 \text{ mmol.L}^{-1}$ ) thus

659 providing a sufficient concentration to inhibit the growth of *E. coli* and *S. aureus* in LB and TSB,  
 660 respectively.

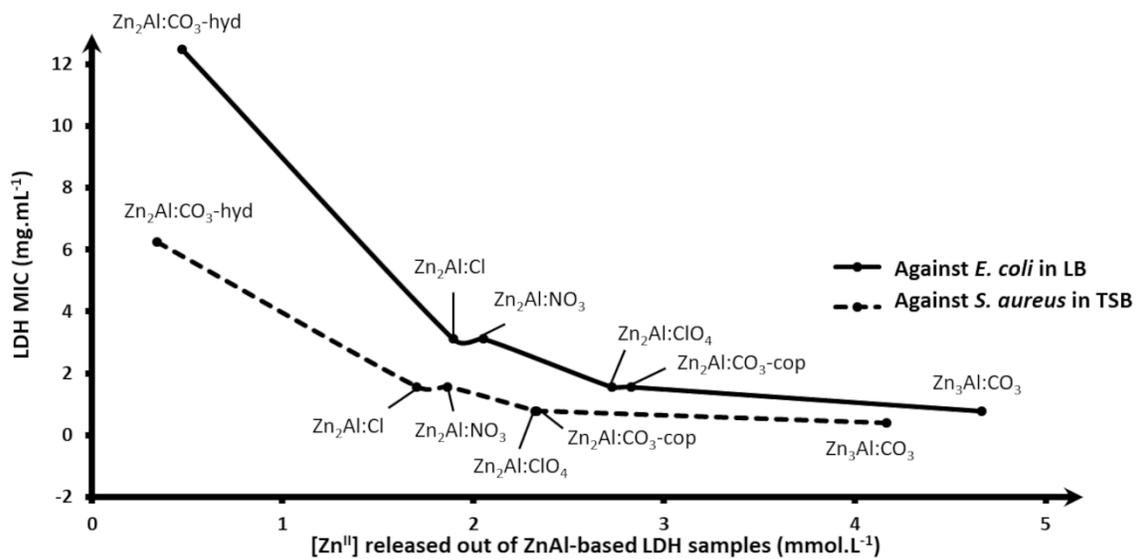
661 **Table 3.** Antimicrobial performance of pristine LDHs and the proposed antibacterial mechanisms by the literature.

Pristine LDH	Tested bacteria	Inhibition		Antimicrobial Performance	Proposed mechanism	Reference
		zone diameter (mm)	MIC (mg.mL <sup>-1</sup> )			
Zn <sub>2</sub> Al:CO <sub>3</sub> Zn <sub>2</sub> Al:Cl Zn <sub>2</sub> Al:NO <sub>3</sub> Zn <sub>2</sub> Al:ClO <sub>4</sub> Zn <sub>3</sub> Al:CO <sub>3</sub> Cu <sub>2</sub> Al:CO <sub>3</sub> Co <sub>2</sub> Al:CO <sub>3</sub> Cu <sub>2</sub> Al:CO <sub>3</sub> Ni <sub>2</sub> Al:CO <sub>3</sub> Mg <sub>2</sub> Al:CO <sub>3</sub>	Gram-positive: <i>Staphylococcus aureus</i> Gram-negative: <i>Escherichia coli</i>	7-13	0.375-12	Depending on the nature of divalent metal (highest activity for Zn <sup>II</sup> -based LDHs) and amount of released Zn <sup>II</sup> in broth media initially dependent on different parameters (crystallinity, Zn <sup>II</sup> :Al <sup>III</sup> molar ratio, nature of intercalating anion)	Metal ion release	<b>This study</b>
Zn <sub>2</sub> Al:CO <sub>3</sub>	Gram-positive: <i>Staphylococcus aureus</i> , <i>Streptococcus lactis</i> , <i>Bacillus cereus</i> Gram-negative: <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Salmonella typhimurium</i>	9-21	0.15-0.3	Depending on the applied LDH dosage and type of tested bacteria	Surface interactions, generation of ROS, metal ions release	<b>(Dutta et al., 2020)</b>
Cu <sub>2</sub> Al:CO <sub>3</sub> Ni <sub>2</sub> Al:CO <sub>3</sub> Co <sub>2</sub> Al:CO <sub>3</sub> Mn <sub>2</sub> Al:CO <sub>3</sub> Mg <sub>2</sub> Al:CO <sub>3</sub>	Gram-positive: <i>Staphylococcus aureus</i> Gram-negative: <i>Escherichia coli</i>	>10	0.8-1.5	Depending on the type of divalent metal: highest activity for Mn <sub>2</sub> Al:CO <sub>3</sub> and Cu <sub>2</sub> Al:CO <sub>3</sub> , minimal activity for Ni <sub>2</sub> Al:CO <sub>3</sub> and Co <sub>2</sub> Al:CO <sub>3</sub> , no activity for Mg <sub>2</sub> Al:CO <sub>3</sub>	Surface interactions, generation of ROS, metal ions release	<b>(M. Li et al., 2020)</b>
Ternary CuMgAl:CO <sub>3</sub>	Gram-positive: <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i> , <i>Bacillus Subtilis</i> Gram-negative: <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	–	0-31	Increasing antibacterial activity with increasing Cu <sup>II</sup> :Al <sup>III</sup> molar ratio	Surface interactions, generation of ROS	<b>(Tabti et al., 2020)</b>

Zn <sub>2</sub> Al:CO <sub>3</sub> Zn <sub>3</sub> Al:CO <sub>3</sub> Zn <sub>4</sub> Al:CO <sub>3</sub>	Gram-positive: <i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> Gram-negative : <i>Pseudomonas aeruginosa</i>	–	4-10	Increasing antibacterial activity with increasing Zn <sup>II</sup> :Al <sup>II</sup> molar ratio	Metal ions release, generation of Reactive Oxygen Species (ROS)	(Lobo-Sánchez et al., 2018)
Ternary CuZnAl:SO <sub>4</sub>	Gram-positive: <i>Staphylococcus aureus</i> Gram-negative: <i>Escherichia coli</i>	8-15	100-310	Depending on the applied LDH dosage	Surface interactions, metal ions release	(Mishra et al., 2018)
Zn <sub>2</sub> Fe:NO <sub>3</sub>	Gram-positive: <i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> Gram-negative: <i>Proteous vulgaris</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	16.8-22.9	–	Depending on the type of tested bacteria and selectivity of LDHs toward different bacterial strains	(Moaty et al., 2016)	(Moaty et al., 2016)

662 Furthermore, the relationship between the solubility of Zn<sup>II</sup>-based LDH in bacterial growth  
663 media and their antimicrobial properties was established. For this purpose, the evolution of the  
664 minimum inhibitory concentration (MIC) of Zn<sup>II</sup>-based LDH samples determined in liquid LB  
665 and TSB broths, was studied as a function of the percentage of released amount of Zn<sup>II</sup> out of  
666 them, *i.e.* Zn<sub>2</sub>Al:CO<sub>3</sub>-hyd, Zn<sub>2</sub>Al:CO<sub>3</sub>-cop, Zn<sub>3</sub>Al:CO<sub>3</sub>, Zn<sub>2</sub>Al:Cl, Zn<sub>2</sub>Al:NO<sub>3</sub>, Zn<sub>2</sub>Al:ClO<sub>4</sub>,  
667 prepared using different experimental procedures. According to **Figure 8**, the obtained MIC  
668 values ranged from 0.375 to 12 mg.mL<sup>-1</sup> depending on the type of Zn<sup>II</sup>-based LDH used and the  
669 amount of zinc ions it released. The obtained MIC values fall within the range of the obtained  
670 MIC values for most pristine antimicrobial LDHs found in the literature with some slight  
671 differences depending on the way the MIC was evaluated and the properties of tested LDHs (M.  
672 Li et al., 2020; Lobo-Sánchez et al., 2018; Z. Wang et al., 2018). The MIC values of Zn<sup>II</sup>-  
673 based LDH samples decreased significantly depending on the amount of released Zn<sup>II</sup> in broth

674 media (**Figure 8**). This points out the impact of the dissolution phenomenon of  $Zn^{II}$ - $Al^{III}$  LDH  
 675 powders on their antibacterial efficiency, by which the antimicrobial activity is enhanced with  
 676 increasing solubility and divalent metal release profiles of their constituent divalent metal. This  
 677 finding supports the possible mechanism of the antibacterial activity of LDHs induced by the  
 678 release of their constituent metallic ions. In other words, when present in aqueous solutions,  $Zn^{II}$ -  
 679 containing LDHs release  $Zn^{II}$  into the medium, where it can act as an inorganic antibacterial  
 680 agent, thus inhibiting the growth of the bacteria.  $Zn^{II}$  soluble species then may damage the  
 681 bacterial membrane function through changing its charge distribution thus blocking the transport  
 682 of substances in and out of the membrane or else by binding to nucleic acids leading to bacterial  
 683 proliferation inhibition (**Peng et al., 2018**).



684  
 685 **Figure 8.** Evolution of antimicrobial activity in terms of LDH MIC against *S. aureus* and *E. coli* as a function of  
 686 average dissolution of  $Zn^{II}$  out of Zn-based LDH samples.

687 It is also remarkable here to mention that the antibacterial efficiencies of both LDH powders  
 688 dispersions and metallic ion salts were more pronounced against *S. aureus* than *E. coli*, although  
 689 the release profile of divalent metals was higher in LB medium than in TSB. This could be

690 simply linked to the specificity of the antimicrobial agents toward a certain bacterial species and  
691 the unlike cell structures of the tested microorganisms, where unlike Gram-positive *S. aureus*  
692 bacteria, Gram-negative *E. coli* bacteria possess an outer membrane and a thick cell wall that  
693 result in a rigid outer coating (Qiao et al., 2018).

#### 694 4. Conclusion

695 In summary, divalent metal release out of pristine LDHs in broth culture media is strongly  
696 dependent on their physicochemical properties as their crystallinity,  $M^{II}:Al^{III}$  ratio, type of  
697 intercalated anion and nature of  $M^{II}$  cations. Lower crystallinity  $Zn_2Al:CO_3$  LDH unexposed to  
698 hydrothermal treatment showed more defects and small aggregated particles and thus released  
699 more zinc ions in broth media. Increasing  $M^{II}:Al^{III}$  ratio in  $Zn^{II}$ -based LDH had also led an  
700 enhanced release of  $Zn^{II}$  ions probably due to the increase of amount of zinc initially present in  
701 the LDH structure and/or due to the reduction in crystallinity. Moreover, the crystallinity and  
702 structure of the powders were also influenced by the anionic exchange reactions which aimed at  
703 eliminating carbonate anions and exchanging them with other intercalating anions, thus resulting  
704 in an amplified release of ions as well. On the other hand, the dissolution properties of  $M^{II}-Al^{III}$   
705 LDHs having different constituent divalent metals ( $M^{II}$ ) is related to the thermodynamic stability  
706 of their  $M^{II}(OH)_2$  hydroxide counterparts, with the exception of  $Cu^{II}$ -based LDHs which released  
707 a higher amount of  $Cu^{II}$ , probably due to its very poor crystallinity and irregular structure  
708 presenting defects. Finally, the antibacterial activity of the synthesized pristine LDHs was  
709 determined, where only  $Zn^{II}$  and  $Cu^{II}$ -based LDHs were able to demonstrate an inhibitory effect  
710 against *E. coli* and *S. aureus*. The superior antimicrobial activity of  $Zn^{II}$ -based LDHs even at  
711 lower release profiles suggest that the antimicrobial effect of pristine LDHs is dependent on the

712 nature of constituent divalent metal on the first place. In addition, the minimum inhibitory  
713 concentration (MIC), required to inhibit the growth of *E. coli* and *S. aureus*, of Zn<sup>II</sup>-based LDHs  
714 decreased with the increasing released Zn<sup>II</sup> amount which was initially controlled by the different  
715 studied physicochemical parameters, *e.g.* crystallinity of the LDHs, Zn<sup>II</sup>:Al<sup>III</sup> molar ratio and  
716 nature of intercalated anion. This provides an evidence about the tight relationship between  
717 divalent metal release out of LDHs and their antimicrobial activity behavior, thus supporting  
718 and validating the suggested mechanism for the antibacterial effect of pristine LDHs by metallic  
719 ions release. Divalent metal species could be leached out of the LDH, *e.g.*, Zn<sup>II</sup> and Cu<sup>II</sup> in this  
720 case, and then might interact in a way or another with the bacterial cell wall or bacterial  
721 compositional DNA to inhibit their growth. Therefore, by controlling the nature of constituent  
722 divalent metal and the different physicochemical parameters responsible for divalent metal  
723 release, pristine LDHs could serve as potential candidates in several antibacterial applications as  
724 antimicrobial coatings, *e.g.* dressing for protecting wounds, medical tools, protection against  
725 biocorrosion and cement additives.

## 726 **Acknowledgment**

727 We would like to acknowledge the spectroscopy and microscopy Service Facility of SMI  
728 LCPME (Université de Lorraine-CNRS– <http://www.lcpme.cnrs-nancy.fr>). Claire Genois from  
729 LCPME (Université de Lorraine-CNRS– <http://www.lcpme.cnrs-nancy.fr>) is particularly  
730 acknowledged for the ICP-MS analysis. We would also like to thank the plateforme PMD<sup>2</sup>X de  
731 l'Institut Jean Barriol de l'Université de Lorraine, in particular Pierrick Durand for the PXRD  
732 analysis. We thank Celine Caillet and Audrey Beaussart from the Pôle de compétences Physico-

733 Chimie de l'Environnement and the PhySI research team of LIEC (Université de Lorraine-  
734 CNRS, <http://liec.univ-lorraine.fr/>) for their help in the PSD analyses.

### 735 **Funding**

736 Financial support was received from the French Ministry of Higher Education (MESR), the  
737 French National Scientific Centre (CNRS). Jazia Awassa acknowledges the French Ministry of  
738 Higher Education for PhD grant.

### 739 **References**

740 Ahmed, A. A. A., Talib, Z. A., & Hussein, M. Z. (2013). Influence of Metallic Molar Ratio on  
741 the Electron Spin Resonance and Thermal Diffusivity of Zn–Al Layered Double  
742 Hydroxide. *Journal of Nanomaterials*, 2013, 1–9. <https://doi.org/10.1155/2013/639354>

743 Allada, R. k. (2002). Thermochemistry and Aqueous Solubilities of Hydrotalcite-Like Solids.  
744 *Science*, 296(5568), 721–723. <https://doi.org/10.1126/science.1069797>

745 Allada, R. K., Pless, J. D., Nenoff, T. M., & Navrotsky, A. (2005). Thermochemistry of  
746 Hydrotalcite-like Phases Intercalated with  $\text{CO}_3^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{I}^-$ , and  $\text{ReO}_4^-$ .  
747 *Chemistry of Materials*, 17(9), 2455–2459. <https://doi.org/10.1021/cm047813x>

748 Baek, M., Kim, I.-S., Yu, J., Chung, H. E., Choy, J.-H., & Choi, S.-J. (2011). Effect of Different  
749 Forms of Anionic Nanoclays on Cytotoxicity. *Journal of Nanoscience and*  
750 *Nanotechnology*, 11(2), 1803–1806. <https://doi.org/10.1166/jnn.2011.3408>

751 Bélteky, P., Rónavári, A., Igaz, N., Szerencsés, B., Tóth, I. Y., Pfeiffer, I., Kiricsi, M., & Kónya,  
752 Z. (2019). Silver nanoparticles: Aggregation behavior in biorelevant conditions and its  
753 impact on biological activity. *International Journal of Nanomedicine*, Volume 14, 667–  
754 687. <https://doi.org/10.2147/IJN.S185965>

755 Benito, P., Labajos, F. M., & Rives, V. (2006). Microwave-treated layered double hydroxides  
756 containing Ni<sup>2+</sup> and Al<sup>3+</sup>: The effect of added Zn<sup>2+</sup>. *Journal of Solid State Chemistry*,  
757 *179*(12), 3784–3797. <https://doi.org/10.1016/j.jssc.2006.08.010>

758 Bi, X., Zhang, H., & Dou, L. (2014). Layered Double Hydroxide-Based Nanocarriers for Drug  
759 Delivery. *Pharmaceutics*, *6*(2), 298–332. <https://doi.org/10.3390/pharmaceutics6020298>

760 Bini, M., & Monteforte, F. (2018). Layered Double Hydroxides (LDHs): Versatile and Powerful  
761 Hosts for Different Applications. *Journal of Analytical & Pharmaceutical Research*,  
762 *7*(1). <https://doi.org/10.15406/japlr.2018.07.00206>

763 Blais, J. F., Djedidi, Z., Cheikh, R. B., Tyagi, R. D., & Mercier, G. (2008). Metals Precipitation  
764 from Effluents: Review. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste*  
765 *Management*, *12*(3), 135–149. [https://doi.org/10.1061/\(ASCE\)1090-](https://doi.org/10.1061/(ASCE)1090-025X(2008)12:3(135))  
766 [025X\(2008\)12:3\(135\)](https://doi.org/10.1061/(ASCE)1090-025X(2008)12:3(135))

767 Boclair, J. W., & Braterman, P. S. (1999). Layered Double Hydroxide Stability. 1. Relative  
768 Stabilities of Layered Double Hydroxides and Their Simple Counterparts. *Chemistry of*  
769 *Materials*, *11*(2), 298–302. <https://doi.org/10.1021/cm980523u>

770 Bontchev, R. P., Liu, S., Krumhansl, J. L., Voigt, J., & Nenoff, T. M. (2003). Synthesis,  
771 Characterization, and Ion Exchange Properties of Hydrotalcite Mg<sub>6</sub>Al<sub>2</sub>(OH)<sub>16</sub>(A)<sub>x</sub>  
772 (A')<sub>2-x</sub>·4H<sub>2</sub>O (A, A' = Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>, 2 ≥ x ≥ 0) Derivatives. *Chemistry*  
773 *of Materials*, *15*(19), 3669–3675. <https://doi.org/10.1021/cm034231r>

774 Borkow, G., & Gabbay, J. (2005). Copper as a Biocidal Tool. *Current Medicinal Chemistry*,  
775 *12*(18), 2163–2175. <https://doi.org/10.2174/0929867054637617>

776 Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial Resistance to Metals in the  
777 Environment. *Ecotoxicology and Environmental Safety*, 45(3), 198–207.  
778 <https://doi.org/10.1006/eesa.1999.1860>

779 Carja, G., Kameshima, Y., Nakajima, A., Dranca, C., & Okada, K. (2009). Nanosized silver–  
780 anionic clay matrix as nanostructured ensembles with antimicrobial activity.  
781 *International Journal of Antimicrobial Agents*, 34(6), 534–539.  
782 <https://doi.org/10.1016/j.ijantimicag.2009.08.008>

783 Cavani, F., Trifirò, F., & Vaccari, A. (1991). Hydrotalcite-type anionic clays: Preparation,  
784 properties and applications. *Catalysis Today*, 11(2), 173–301.  
785 [https://doi.org/10.1016/0920-5861\(91\)80068-K](https://doi.org/10.1016/0920-5861(91)80068-K)

786 Cheng, H.-M., Gao, X.-W., Zhang, K., Wang, X.-R., Zhou, W., Li, S.-J., Cao, X.-L., & Yan, D.-  
787 P. (2019). A novel antimicrobial composite: ZnAl-hydrotalcite with *p*-hydroxybenzoic  
788 acid intercalation and its possible application as a food packaging material. *New Journal*  
789 *of Chemistry*, 43(48), 19408–19414. <https://doi.org/10.1039/C9NJ03943K>

790 Clark, I., Gomes, R. L., Crawshaw, C., Neve, L., Lodge, R., Fay, M., Winkler, C., Hull, M., &  
791 Lester, E. (2019). Continuous synthesis of Zn<sub>2</sub>Al–CO<sub>3</sub> layered double hydroxides: A  
792 comparison of bench, pilot and industrial scale syntheses. *Reaction Chemistry &*  
793 *Engineering*, 4(4), 663–666. <https://doi.org/10.1039/C8RE00241J>

794 Crespo, I. (1997). Intercalation of iron hexacyano complexes in Zn,Al-hydrotalcite. *Solid State*  
795 *Ionics*, 101–103, 729–735. [https://doi.org/10.1016/S0167-2738\(97\)00290-7](https://doi.org/10.1016/S0167-2738(97)00290-7)

796 Cullity, B. D. (1978). *Elements of x-ray diffraction* (2d ed). Addison-Wesley Pub. Co.

797 Djebbi, M. A., Elabed, A., Bouaziz, Z., Sadiki, M., Elabed, S., Namour, P., Jaffrezic-Renault, N.,  
798 & Amara, A. B. H. (2016). Delivery system for berberine chloride based on the

799 nanocarrier ZnAl-layered double hydroxide: Physicochemical characterization, release  
800 behavior and evaluation of anti-bacterial potential. *International Journal of*  
801 *Pharmaceutics*, 515(1–2), 422–430. <https://doi.org/10.1016/j.ijpharm.2016.09.089>

802 Dutta, S., Jana, T. K., Halder, S. K., Maiti, R., Dutta, A., Kumar, A., & Chatterjee, K. (2020). Zn  
803 <sub>2</sub> Al- CO <sub>3</sub> Layered Double Hydroxide: Adsorption, Cytotoxicity and Antibacterial  
804 Performances. *ChemistrySelect*, 5(20), 6162–6171.  
805 <https://doi.org/10.1002/slct.202001264>

806 El-Shahawy, A. A. G., Abo El-Ela, F. I., Mohamed, N. A., Eldine, Z. E., & El Rouby, W. M. A.  
807 (2018). Synthesis and evaluation of layered double hydroxide/doxycycline and cobalt  
808 ferrite/chitosan nanohybrid efficacy on gram positive and gram negative bacteria.  
809 *Materials Science and Engineering: C*, 91, 361–371.  
810 <https://doi.org/10.1016/j.msec.2018.05.042>

811 Esteban-Tejeda, L., Palomares, F., Cabal, B., López-Píriz, R., Fernández, A., Sevillano, D.,  
812 Alou, L., Torrecillas, R., & Moya, J. (2017). Effect of the Medium Composition on the  
813 Zn<sup>2+</sup> Lixiviation and the Antifouling Properties of a Glass with a High ZnO Content.  
814 *Materials*, 10(2), 167. <https://doi.org/10.3390/ma10020167>

815 Forano, C., Bruna, F., Mousty, C., & Prevot, V. (2018). Interactions between Biological Cells  
816 and Layered Double Hydroxides: Towards Functional Materials. *The Chemical Record*,  
817 18(7–8), 1150–1166. <https://doi.org/10.1002/tcr.201700102>

818 Galvão, T. L. P., Neves, C. S., Caetano, A. P. F., Maia, F., Mata, D., Malheiro, E., Ferreira, M.  
819 J., Bastos, A. C., Salak, A. N., Gomes, J. R. B., Tedim, J., & Ferreira, M. G. S. (2016).  
820 Control of crystallite and particle size in the synthesis of layered double hydroxides:

821 Macromolecular insights and a complementary modeling tool. *Journal of Colloid and*  
822 *Interface Science*, 468, 86–94. <https://doi.org/10.1016/j.jcis.2016.01.038>

823 Herrero, M., Labajos, F., & Rives, V. (2008). Size control and optimisation of intercalated  
824 layered double hydroxides. *Applied Clay Science*, S0169131708001336.  
825 <https://doi.org/10.1016/j.clay.2008.06.011>

826 Hibino, T. (2014). Acid Treatment of Layered Double Hydroxides Containing Carbonate: Acid  
827 Treatment of Layered Double Hydroxides Containing Carbonate. *European Journal of*  
828 *Inorganic Chemistry*, 2014(31), 5311–5321. <https://doi.org/10.1002/ejic.201402372>

829 Inmauddin (Ed.). (2019). Layered double Hydroxide Based Biosensors. In *Materials Research*  
830 *Foundations* (1st ed., Vol. 47, pp. 131–156). Materials Research Forum LLC.  
831 <https://doi.org/10.21741/9781644900130-4>

832 Iyi, N., Yamada, H., & Sasaki, T. (2011). Deintercalation of carbonate ions from carbonate-type  
833 layered double hydroxides (LDHs) using acid–alcohol mixed solutions. *Applied Clay*  
834 *Science*, 54(2), 132–137. <https://doi.org/10.1016/j.clay.2011.07.017>

835 Jobbágy, M., & Regazzoni, A. E. (2011). Dissolution of nano-size Mg–Al–Cl hydrotalcite in  
836 aqueous media. *Applied Clay Science*, 51(3), 366–369.  
837 <https://doi.org/10.1016/j.clay.2010.11.027>

838 Johnston, A.-L., Lester, E., Williams, O., & Gomes, R. L. (2021). Understanding Layered  
839 Double Hydroxide properties as sorbent materials for removing organic pollutants from  
840 environmental waters. *Journal of Environmental Chemical Engineering*, 9(4), 105197.  
841 <https://doi.org/10.1016/j.jece.2021.105197>

842 Kamal, W., El Rouby, W. M. A., El-Gendy, A. O., & Farghali, A. A. (2018). Bimodal  
843 applications of LDH-chitosan nanocomposite: Water treatment and antimicrobial activity.

844 *IOP Conference Series: Materials Science and Engineering*, 464, 012005.  
845 <https://doi.org/10.1088/1757-899X/464/1/012005>

846 Kannan, S., Rives, V., & Knözinger, H. (2004). High-temperature transformations of Cu-rich  
847 hydroxalicates. *Journal of Solid State Chemistry*, 177(1), 319–331.  
848 <https://doi.org/10.1016/j.jssc.2003.08.023>

849 Kim, S., Fahel, J., Durand, P., André, E., & Carteret, C. (2017). Ternary Layered Double  
850 Hydroxides (LDHs) Based on Co-, Cu-Substituted ZnAl for the Design of Efficient  
851 Photocatalysts: Ternary Layered Double Hydroxides (LDHs) Based on Co-, Cu-  
852 Substituted ZnAl for the Design of Efficient Photocatalysts. *European Journal of*  
853 *Inorganic Chemistry*, 2017(3), 669–678. <https://doi.org/10.1002/ejic.201601213>

854 Kloprogge, J. T., Wharton, D., Hickey, L., & Frost, R. L. (2002). Infrared and Raman study of  
855 interlayer anions  $\text{CO}_3^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$  and  $\text{ClO}_4^-$  in Mg/Al-hydroxalcite. *American*  
856 *Mineralogist*, 87(5–6), 623–629. <https://doi.org/10.2138/am-2002-5-604>

857 Kovanda, F., Rojka, T., Bezdička, P., Jirátová, K., Obalová, L., Pacultová, K., Bastl, Z., &  
858 Grygar, T. (2009). Effect of hydrothermal treatment on properties of Ni–Al layered  
859 double hydroxides and related mixed oxides. *Journal of Solid State Chemistry*, 182(1),  
860 27–36. <https://doi.org/10.1016/j.jssc.2008.09.014>

861 Lee, J.-Y., Gwak, G.-H., Kim, H.-M., Kim, T., Lee, G. J., & Oh, J.-M. (2016). Synthesis of  
862 hydroxalcite type layered double hydroxide with various Mg/Al ratio and surface charge  
863 under controlled reaction condition. *Applied Clay Science*, 134, 44–49.  
864 <https://doi.org/10.1016/j.clay.2016.03.029>

865 León-Vallejo, A. M., Velázquez-Herrera, F. D., Sampieri, Á., Landeta-Cortés, G., & Fetter, G.  
866 (2019). Study of layered double hydroxides as bactericidal materials against

867 Corynebacterium ammoniagenes, a bacterium responsible for producing bad odors from  
868 human urine and skin infections. *Applied Clay Science*, 180, 105194.  
869 <https://doi.org/10.1016/j.clay.2019.105194>

870 Li, J., Zhang, S., Chen, Y., Liu, T., Liu, C., Zhang, X., Yi, M., Chu, Z., & Han, X. (2017). A  
871 novel three-dimensional hierarchical CuAl layered double hydroxide with excellent  
872 catalytic activity for degradation of methyl orange. *RSC Advances*, 7(46), 29051–29057.  
873 <https://doi.org/10.1039/C7RA03848H>

874 Li, M., Li, L., & Lin, S. (2020). Efficient antimicrobial properties of layered double hydroxide  
875 assembled with transition metals via a facile preparation method. *Chinese Chemical*  
876 *Letters*, 31(6), 1511–1515. <https://doi.org/10.1016/j.ccllet.2019.09.047>

877 Li, M., Xu, Z. P., Sultanbawa, Y., Chen, W., Liu, J., & Qian, G. (2019). Potent and durable  
878 antibacterial activity of ZnO-dotted nanohybrids hydrothermally derived from ZnAl-  
879 layered double hydroxides. *Colloids and Surfaces B: Biointerfaces*, 181, 585–592.  
880 <https://doi.org/10.1016/j.colsurfb.2019.06.013>

881 Li, M., Zhu, L., & Lin, D. (2011). Toxicity of ZnO Nanoparticles to *Escherichia coli*:  
882 Mechanism and the Influence of Medium Components. *Environmental Science &*  
883 *Technology*, 45(5), 1977–1983. <https://doi.org/10.1021/es102624t>

884 Lieth, R. M. A. (Ed.). (1977). *Preparation and Crystal Growth of Materials with Layered*  
885 *Structures*. Springer Netherlands. <https://doi.org/10.1007/978-94-017-2750-1>

886 Liu, H., Jiao, Q., Zhao, Y., Li, H., Sun, C., & Li, X. (2010). Mixed oxides derived from Cu–Co  
887 layered double hydroxide nanorods: Preparation, characterization and their catalytic  
888 activities. *Journal of Alloys and Compounds*, 496(1–2), 317–323.  
889 <https://doi.org/10.1016/j.jallcom.2010.02.004>

890 Liu, S., Killen, E., Lim, M., Gunawan, C., & Amal, R. (2014). The effect of common bacterial  
891 growth media on zinc oxide thin films: Identification of reaction products and  
892 implications for the toxicology of ZnO. *RSC Adv.*, 4(9), 4363–4370.  
893 <https://doi.org/10.1039/C3RA46177G>

894 Liu, Y., & Yang, Z. (2016). Intercalation of sulfate anions into a Zn–Al layered double  
895 hydroxide: Their synthesis and application in Zn–Ni secondary batteries. *RSC Advances*,  
896 6(73), 68584–68591. <https://doi.org/10.1039/C6RA09096F>

897 Lobo-Sánchez, M., Nájera-Meléndez, G., Luna, G., Segura-Pérez, V., Rivera, J. A., & Fetter, G.  
898 (2018). ZnAl layered double hydroxides impregnated with eucalyptus oil as efficient  
899 hybrid materials against multi-resistant bacteria. *Applied Clay Science*, 153, 61–69.  
900 <https://doi.org/10.1016/j.clay.2017.11.017>

901 Mahjoubi, F. Z., Khalidi, A., Abdennouri, M., & Barka, N. (2017). Zn–Al layered double  
902 hydroxides intercalated with carbonate, nitrate, chloride and sulphate ions: Synthesis,  
903 characterisation and dye removal properties. *Journal of Taibah University for Science*,  
904 11(1), 90–100. <https://doi.org/10.1016/j.jtusc.2015.10.007>

905 Mallakpour, S., Radfar, Z., & Hussain, C. M. (2021). Current advances on polymer-layered  
906 double hydroxides/metal oxides nanocomposites and bionanocomposites: Fabrications  
907 and applications in the textile industry and nanofibers. *Applied Clay Science*, 206,  
908 106054. <https://doi.org/10.1016/j.clay.2021.106054>

909 Mao, L., Liu, J., Zheng, S., Wu, H., Liu, Y., Li, Z., & Bai, Y. (2019). Mussel-inspired nano-  
910 silver loaded layered double hydroxides embedded into a biodegradable polymer matrix  
911 for enhanced mechanical and gas barrier properties. *RSC Advances*, 9(10), 5834–5843.  
912 <https://doi.org/10.1039/C8RA09602C>

913 Marcato, P. D., Parizotto, N. V., Martinez, D. S. T., Paula, A. J., Ferreira, I. R., Melo, P. S.,  
914 Durán, N., & Alves, O. L. (2013). New Hybrid Material Based on Layered Double  
915 Hydroxides and Biogenic Silver Nanoparticles: Antimicrobial Activity and Cytotoxic  
916 Effect. *Journal of the Brazilian Chemical Society*, 24(2), 266–272.  
917 <https://doi.org/10.5935/0103-5053.20130034>

918 Mishra, G., Dash, B., Pandey, S., & Mohanty, P. P. (2013). Antibacterial actions of silver  
919 nanoparticles incorporated Zn–Al layered double hydroxide and its spinel. *Journal of*  
920 *Environmental Chemical Engineering*, 1(4), 1124–1130.  
921 <https://doi.org/10.1016/j.jece.2013.08.031>

922 Mishra, G., Dash, B., Pandey, S., & Sethi, D. (2018). Ternary layered double hydroxides (LDH)  
923 based on Cu- substituted Zn Al for the design of efficient antibacterial ceramics. *Applied*  
924 *Clay Science*, 165, 214–222. <https://doi.org/10.1016/j.clay.2018.08.021>

925 Mittapally, S., Taranum, R., & Parveen, S. (2018). Metal ions as antibacterial agents. *Journal of*  
926 *Drug Delivery and Therapeutics*, 8(6-s), 411–419. [https://doi.org/10.22270/jddt.v8i6-](https://doi.org/10.22270/jddt.v8i6-s.2063)  
927 [s.2063](https://doi.org/10.22270/jddt.v8i6-s.2063)

928 Miyata, S. (1980). Physico-Chemical Properties of Synthetic Hydrotalcites in Relation to  
929 Composition. *Clays and Clay Minerals*, 28(1), 50–56.  
930 <https://doi.org/10.1346/CCMN.1980.0280107>

931 Moaty, S. A. A., Farghali, A. A., & Khaled, R. (2016). Preparation, characterization and  
932 antimicrobial applications of Zn-Fe LDH against MRSA. *Materials Science and*  
933 *Engineering: C*, 68, 184–193. <https://doi.org/10.1016/j.msec.2016.05.110>

934 Pasquet, J., Chevalier, Y., Pelletier, J., Couval, E., Bouvier, D., & Bolzinger, M.-A. (2014). The  
935 contribution of zinc ions to the antimicrobial activity of zinc oxide. *Colloids and Surfaces*

936 A: *Physicochemical and Engineering Aspects*, 457, 263–274.  
937 <https://doi.org/10.1016/j.colsurfa.2014.05.057>

938 Pavlovic, M. (2016). Ion specific effects on the stability of layered double hydroxide colloids.  
939 *Soft Matter*, 10.

940 Peng, F., Wang, D., Zhang, D., Cao, H., & Liu, X. (2018). The prospect of layered double  
941 hydroxide as bone implants: A study of mechanical properties, cytocompatibility and  
942 antibacterial activity. *Applied Clay Science*, 165, 179–187.  
943 <https://doi.org/10.1016/j.clay.2018.08.020>

944 Prasanna, S. V., & Kamath, P. V. (2009). Anion-Exchange Reactions of Layered Double  
945 Hydroxides: Interplay between Coulombic and H-Bonding Interactions. *Industrial &*  
946 *Engineering Chemistry Research*, 48(13), 6315–6320. <https://doi.org/10.1021/ie9004332>

947 Qiao, Y., Li, Q., Chi, H., Li, M., Lv, Y., Feng, S., Zhu, R., & Li, K. (2018). Methyl blue  
948 adsorption properties and bacteriostatic activities of Mg-Al layer oxides via a facile  
949 preparation method. *Applied Clay Science*, 163, 119–128.  
950 <https://doi.org/10.1016/j.clay.2018.07.018>

951 Radha, A. V., Vishnu Kamath, P., & Shivakumara, C. (2005). Mechanism of the anion exchange  
952 reactions of the layered double hydroxides (LDHs) of Ca and Mg with Al. *Solid State*  
953 *Sciences*, 7(10), 1180–1187. <https://doi.org/10.1016/j.solidstatesciences.2005.05.004>

954 Rasouli, N., Movahedi, M., & Doudi, M. (2017). Synthesis and characterization of inorganic  
955 mixed metal oxide nanoparticles derived from Zn–Al layered double hydroxide and their  
956 antibacterial activity. *Surfaces and Interfaces*, 6, 110–115.  
957 <https://doi.org/10.1016/j.surfin.2016.11.007>

958 Rawle, A. (2003). The Basic Principles of Particle Size Analysis. *Surface Coatings International*  
959 *Part A: Coatings Journal*, 86, 58–65.

960 Rives, V. (Ed.). (2001). *Layered double hydroxides: Present and future*. Nova Science  
961 Publishers.

962 Rocha Oliveira, G., Dias do Amaral, L. J., Giovanella, M., da Silva Crespo, J., Fetter, G., Rivera,  
963 J. A., Sampieri, A., & Bosch, P. (2015). Bactericidal Performance of Chlorophyllin-  
964 Copper Hydrotalcite Compounds. *Water, Air, & Soil Pollution*, 226(9), 316.  
965 <https://doi.org/10.1007/s11270-015-2585-1>

966 Rojas, R. (2016). Effect of particle size on copper removal by layered double hydroxides.  
967 *Chemical Engineering Journal*, 303, 331–337. <https://doi.org/10.1016/j.cej.2016.06.007>

968 Salak, A. N., Tedim, J., Kuznetsova, A. I., Zheludkevich, M. L., & Ferreira, M. G. S. (2010).  
969 Anion exchange in Zn–Al layered double hydroxides: In situ X-ray diffraction study.  
970 *Chemical Physics Letters*, 495(1–3), 73–76. <https://doi.org/10.1016/j.cplett.2010.06.041>

971 Salguero, Y., Valenti, L., Rojas, R., & García, M. C. (2020). Ciprofloxacin-intercalated layered  
972 double hydroxide-in-hybrid films as composite dressings for controlled antimicrobial  
973 topical delivery. *Materials Science and Engineering: C*, 111, 110859.  
974 <https://doi.org/10.1016/j.msec.2020.110859>

975 Scarpellini, D., Falconi, C., Gaudio, P., Mattoccia, A., Medaglia, P. G., Orsini, A., Pizzoferrato,  
976 R., & Richetta, M. (2014). Morphology of Zn/Al layered double hydroxide nanosheets  
977 grown onto aluminum thin films. *Microelectronic Engineering*, 126, 129–133.  
978 <https://doi.org/10.1016/j.mee.2014.07.007>

- 979 Sharma, M., & Prasher, P. (2020). Metallic Nanoparticles at the Biointerface. In C. Bhargava &  
980 A. Sachdeva (Eds.), *Nanotechnology* (1st ed., pp. 261–276). CRC Press.  
981 <https://doi.org/10.1201/9781003082859-15>
- 982 Sun, X., & Dey, S. K. (2015). Insights into the synthesis of layered double hydroxide (LDH)  
983 nanoparticles: Part 2. Formation mechanisms of LDH. *Journal of Colloid and Interface*  
984 *Science*, 458, 160–168. <https://doi.org/10.1016/j.jcis.2015.06.025>
- 985 Tabti, H. A., Adjdir, M., Ammam, A., Mdjahed, B., Guezzen, B., Ramdani, A., Bendeddouche,  
986 C. K., Bouchikhi, N., & Chami, N. (2020). Facile synthesis of Cu-LDH with different  
987 Cu/Al molar ratios: Application as antibacterial inhibitors. *Research on Chemical*  
988 *Intermediates*, 46(12), 5377–5390. <https://doi.org/10.1007/s11164-020-04268-8>
- 989 Tammaro, L., Vittoria, V., & Bugatti, V. (2014). Dispersion of modified layered double  
990 hydroxides in Poly(ethylene terephthalate) by High Energy Ball Milling for food  
991 packaging applications. *European Polymer Journal*, 52, 172–180.  
992 <https://doi.org/10.1016/j.eurpolymj.2014.01.001>
- 993 Theiss, F. L., Ayoko, G. A., & Frost, R. L. (2016). Synthesis of layered double hydroxides  
994 containing Mg<sup>2+</sup>, Zn<sup>2+</sup>, Ca<sup>2+</sup> and Al<sup>3+</sup> layer cations by co-precipitation methods—A  
995 review. *Applied Surface Science*, 383, 200–213.  
996 <https://doi.org/10.1016/j.apsusc.2016.04.150>
- 997 Wang, C.-C., & Ying, J. Y. (1999). Sol–Gel Synthesis and Hydrothermal Processing of Anatase  
998 and Rutile Titania Nanocrystals. *Chemistry of Materials*, 11(11), 3113–3120.  
999 <https://doi.org/10.1021/cm990180f>
- 1000 Wang, L., Zhu, Z., Wang, F., Qi, Y., Zhang, W., & Wang, C. (2021). State-of-the-art and  
1001 prospects of Zn-containing layered double hydroxides (Zn-LDH)-based materials for

1002 photocatalytic water remediation. *Chemosphere*, 278, 130367.  
1003 <https://doi.org/10.1016/j.chemosphere.2021.130367>

1004 Wang, Y., & Zhang, D. (2012). Synthesis, characterization, and controlled release antibacterial  
1005 behavior of antibiotic intercalated Mg–Al layered double hydroxides. *Materials Research*  
1006 *Bulletin*, 47(11), 3185–3194. <https://doi.org/10.1016/j.materresbull.2012.08.029>

1007 Wang, Z., Yu, H., Ma, K., Chen, Y., Zhang, X., Wang, T., Li, S., Zhu, X., & Wang, X. (2018).  
1008 Flower-like Surface of Three-Metal-Component Layered Double Hydroxide Composites  
1009 for Improved Antibacterial Activity of Lysozyme. *Bioconjugate Chemistry*, 29(6), 2090–  
1010 2099. <https://doi.org/10.1021/acs.bioconjchem.8b00305>

1011 WHO. (2019). *New report calls for urgent action to avert antimicrobial resistance crisis*. World  
1012 Health Organization. [https://www.who.int/news/item/29-04-2019-new-report-calls-for-](https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis)  
1013 [urgent-action-to-avert-antimicrobial-resistance-crisis](https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis)

1014 Williams, O., Clark, I., Gomes, R. L., Pehinec, T., Hobman, J. L., Stekel, D. J., Hyde, R.,  
1015 Dodds, C., & Lester, E. (2019). Removal of copper from cattle footbath wastewater with  
1016 layered double hydroxide adsorbents as a route to antimicrobial resistance mitigation on  
1017 dairy farms. *Science of The Total Environment*, 655, 1139–1149.  
1018 <https://doi.org/10.1016/j.scitotenv.2018.11.330>

1019 Yang, Z., Zhang, C., Zeng, G., Tan, X., Huang, D., Zhou, J., Fang, Q., Yang, K., Wang, H., Wei,  
1020 J., & Nie, K. (2021). State-of-the-art progress in the rational design of layered double  
1021 hydroxide based photocatalysts for photocatalytic and photoelectrochemical H<sub>2</sub>/O<sub>2</sub>  
1022 production. *Coordination Chemistry Reviews*, 446, 214103.  
1023 <https://doi.org/10.1016/j.ccr.2021.214103>

1024 Zhang, J., Zhang, F., Ren, L., Evans, D. G., & Duan, X. (2004). Synthesis of layered double  
1025 hydroxide anionic clays intercalated by carboxylate anions. *Materials Chemistry and*  
1026 *Physics*, 85(1), 207–214. <https://doi.org/10.1016/j.matchemphys.2004.01.020>

1027 Zhang, W., Zhao, Y., Wang, W., Peng, J., Li, Y., Shanguan, Y., Ouyang, G., Xu, M., Wang, S.,  
1028 Wei, J., Wei, H., Li, W., & Yang, Z. (2020). Colloidal Surface Engineering: Growth of  
1029 Layered Double Hydroxides with Intrinsic Oxidase- Mimicking Activities to Fight  
1030 Against Bacterial Infection in Wound Healing. *Advanced Healthcare Materials*, 9(17),  
1031 2000092. <https://doi.org/10.1002/adhm.202000092>

1032 Zheng, Q., Hao, Y., Ye, P., Guo, L., Wu, H., Guo, Q., Jiang, J., Fu, F., & Chen, G. (2013). A pH-  
1033 responsive controlled release system using layered double hydroxide (LDH)-capped  
1034 mesoporous silica nanoparticles. *Journal of Materials Chemistry B*, 1(11), 1644.  
1035 <https://doi.org/10.1039/c3tb00518f>

1036

1037 **Figures:**

1038 **Figure 1.** PXRD patterns of the different prepared LDH samples: a)  $Zn_2Al:CO_3$ -hyd, b)

1039  $Zn_2Al:CO_3$ -cop, c)  $Zn_3Al:CO_3$ , d)  $Zn_2Al:Cl$ , e)  $Zn_2Al:NO_3$ , f)  $Zn_2Al:ClO_4$ , g)  $Mg_2Al:CO_3$ , h)

1040  $Cu_2Al:CO_3$ , i)  $Ni_2Al:CO_3$ , j)  $Co_2Al:CO_3$ . ..... 15

1041 **Figure 2.** SEM micrographs of: a,b)  $Zn_2Al:CO_3$ -hyd, c,d)  $Zn_2Al:CO_3$ -cop, e,f)  $Zn_3Al:CO_3$ . ..... 21

1042 **Figure 3.** SEM micrographs of  $Cu_2Al:CO_3$  LDH. .... 22

1043 **Figure 4.** Particle Size Distribution (PSD) curves in water dispersion of: a)  $Zn_2Al:CO_3$ -hyd, b)

1044  $Zn_2Al:CO_3$ -cop, c)  $Zn_3Al:CO_3$ , d)  $Zn_2Al:Cl$ , e)  $Zn_2Al:NO_3$ , and f)  $Zn_2Al:ClO_4$ . .... 25

1045 **Figure 5.** Partial dissolution profiles of all synthesized LDH samples into  $M^{II}$  in LB and TSB

1046 media in terms of average percentage compared to the initial amount present in the LDH. .... 26

1047 **Figure 6.** Antibacterial agar disk diffusion tests against: a) *S. aureus* control, b) *S. aureus* using

1048  $Zn_2Al:CO_3$ -hyd (top),  $Zn_2Al:CO_3$ -cop (bottom left) and  $Zn_3Al:CO_3$  (bottom right), c) *S. aureus*

1049 using  $Zn_2Al:Cl$  (top),  $Zn_2Al:NO_3$  (bottom left) and  $Zn_2Al:ClO_4$  (bottom right), d) *E. coli* control,

1050 e) *E. coli* using  $Zn_2Al:CO_3$ -hyd (top),  $Zn_2Al:CO_3$ -cop (bottom left) and  $Zn_3Al:CO_3$  (bottom

1051 right), f) *E. coli* using  $Zn_2Al:Cl$  (top),  $Zn_2Al:NO_3$  (bottom left) and  $Zn_2Al:ClO_4$  (bottom right). 33

1052 **Figure 7.** Antimicrobial activity of LDH samples and metallic ion solutions against *S. aureus*

1053 and *E. coli* in terms of mean zone of inhibition. .... 34

1054 **Figure 8.** Evolution of antimicrobial activity in terms of LDH MIC against *S. aureus* and *E. coli*

1055 as a function of average dissolution of  $Zn^{II}$  out of Zn-based LDH samples. .... 38

1056 **Figure S1.** Infrared spectra (ATR) of  $Zn_2Al$  LDHs with different intercalating anions. .... 57

1057 **Figure S2.** Raman spectra of  $Zn_2Al$  LDHs with different intercalating anions. .... 57

1058

1059

## Supplementary Material

### 1060 Divalent Metal Release and Antimicrobial Effects of Layered Double Hydroxides

1061 Jazia Awassa<sup>1</sup>, Damien Cornu<sup>1</sup>, Samantha Soulé<sup>1</sup>, Cédric Carteret<sup>1</sup>,1062 Christian Ruby<sup>1\*</sup>, Sofiane El-Kirat-Chatel<sup>1</sup>1063 <sup>1</sup>Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement

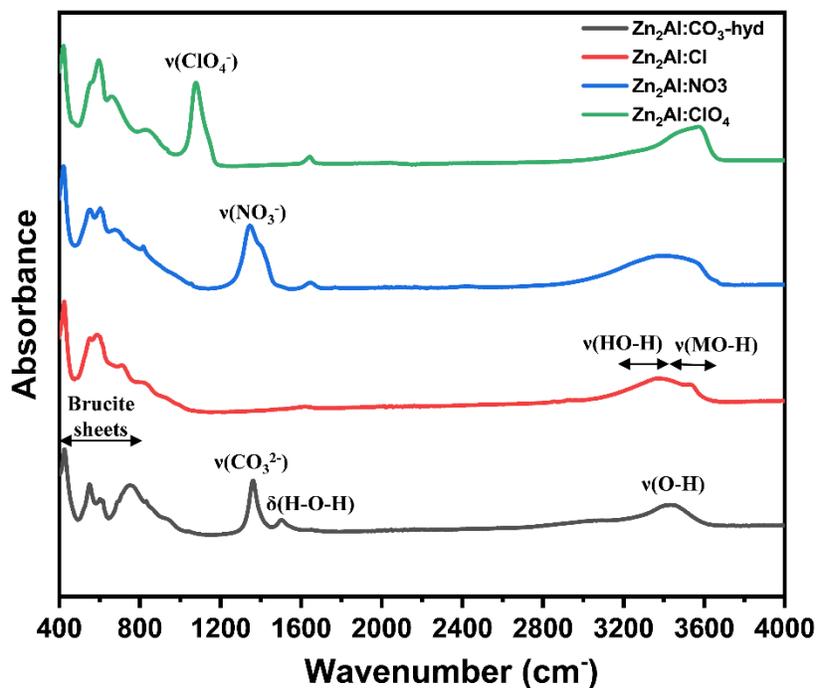
1064 (LCPME), Université de Lorraine, 405 Rue de Vandoeuvre, 54600, Villers-lès-Nancy, France

1065

1066 **Table S1.** Experimental conditions for the synthesis of carbonated LDHs.

LDH sample	M <sup>II</sup> :Al <sup>III</sup>	n(M <sup>II</sup> ) (mol)	n(M <sup>III</sup> ) (mol)	Hydrothermal treatment	M <sup>II</sup> :Al <sup>III</sup> determined by ICP
Zn <sub>2</sub> Al:CO <sub>3</sub> -hyd	2	0.053	0.027	Yes	1.9
Zn <sub>2</sub> Al:CO <sub>3</sub> -cop	2	0.053	0.027	No	1.9
Zn <sub>3</sub> Al:CO <sub>3</sub>	3	0.060	0.020	Yes	2.9
Cu <sub>2</sub> Al:CO <sub>3</sub>	2	0.053	0.027	Yes	2
Ni <sub>2</sub> Al:CO <sub>3</sub>	2	0.053	0.027	Yes	1.9
Co <sub>2</sub> Al:CO <sub>3</sub>	2	0.053	0.027	Yes	2
Mg <sub>2</sub> Al:CO <sub>3</sub>	2	0.053	0.027	Yes	2.1

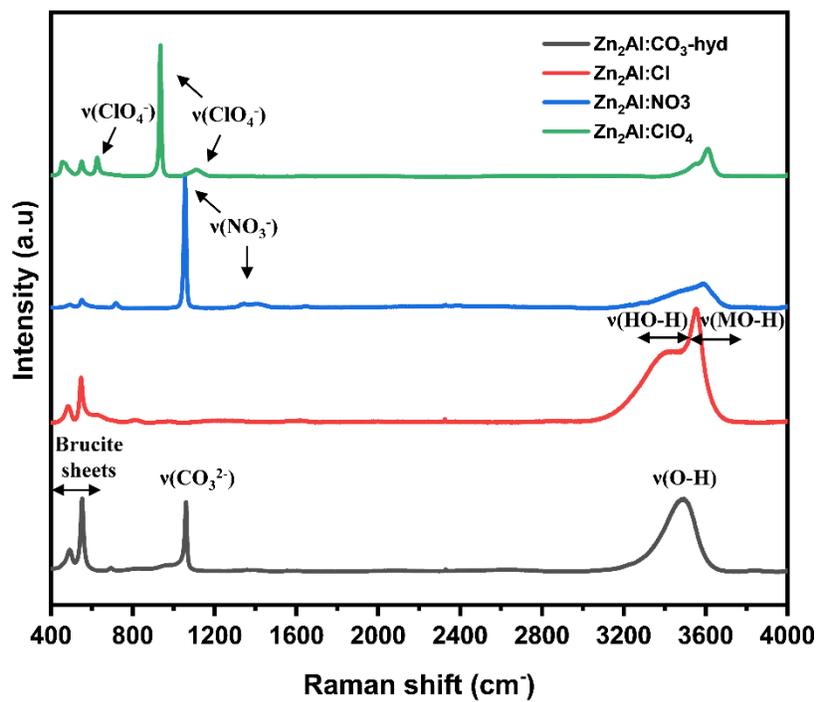
1067



1068

1069

Figure S1. Infrared spectra (ATR) of  $Zn_2Al$  LDHs with different intercalating anions.



1070

1071

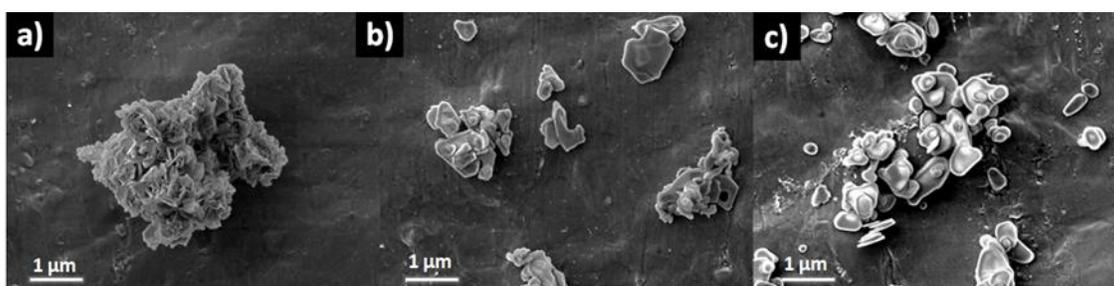
Figure S2. Raman spectra of  $Zn_2Al$  LDHs with different intercalating anions.

1072  
1073

**Table S2.** Partial dissolution profiles of all synthesized LDH samples into  $M^{II}$  in terms of average percentage compared to the initial amount present in the LDH.

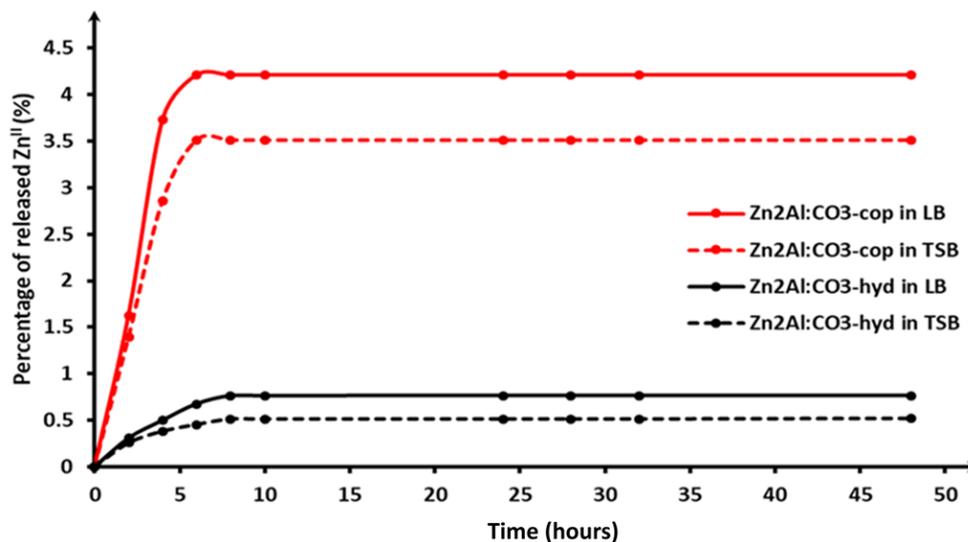
LDH sample	Percentage of released $M^{II}$ (%)
Zn <sub>2</sub> Al:CO <sub>3</sub> -hyd, Zn <sub>2</sub> Al:CO <sub>3</sub> -cop, Zn <sub>3</sub> Al:CO <sub>3</sub> , Zn <sub>2</sub> Al:Cl, Zn <sub>2</sub> Al:NO <sub>3</sub> , Zn <sub>2</sub> Al:ClO <sub>4</sub> , Cu <sub>2</sub> Al:CO <sub>3</sub> , Ni <sub>2</sub> Al:CO <sub>3</sub> , Co <sub>2</sub> Al:CO <sub>3</sub> , Mg <sub>2</sub> Al:CO <sub>3</sub>	Below detection point

1074



1075  
1076

**Figure S3.** SEM micrographs of: a) Zn<sub>2</sub>Al:Cl, b) Zn<sub>2</sub>Al:NO<sub>3</sub>, c) Zn<sub>2</sub>Al:ClO<sub>4</sub>.



1077  
1078

**Figure S4.** Dissolution profiles of Zn<sub>2</sub>Al:CO<sub>3</sub>-hyd and Zn<sub>2</sub>Al:CO<sub>3</sub>-cop over time.

1079

**Table S3.** pK<sub>sp</sub> values for  $M^{II}(\text{OH})_2$  reported by (Blais et al., 2008).

Metal hydroxide	pK <sub>sp</sub>

---

$\text{Zn}^{\text{II}}(\text{OH})_2$	15.7
$\text{Cu}^{\text{II}}(\text{OH})_2$	18.6
$\text{Ni}^{\text{II}}(\text{OH})_2$	14.7
$\text{Co}^{\text{II}}(\text{OH})_2$	14.8
$\text{Mg}^{\text{II}}(\text{OH})_2$	10.8

---

1080