

## Study of the Effect of Acid-base Character of the Lamellar Double Hydroxides "Zn<sub>3</sub>Al-CO<sub>3</sub>" and of the "Ghassoul" Clay on their Redox Potential and Antimicrobial Activities

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**Abstract:** Our study is to highlight the effect of the acid-base character and the redox potential of two clays, synthetic anionic Layered Double Hydroxides Zn<sub>3</sub>Al-CO<sub>3</sub> (LDH) clay, and natural commercial cationic clay "Ghassoul" on their antioxidant and antibacterial activities. The antibacterial effect was tested on two Gram-positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis*. Then it was tested on a Gram-negative bacterium: *Escherichia coli*. The determination of the minimum inhibitory concentration of the two materials was carried out using the microplate microtitration technique. The antioxidant activities of clays are assessed by the methods 2,2-diphenyl-1-picrylhydrazyl and the reducing power of iron (Fe<sup>3+</sup>). The redox potential (Eh) was measured and the redox strength (rH<sub>2</sub>) was evaluated. The results showed that these materials have an antibacterial effect on the three bacteria tested, the measured zero charge point of Ghassoul (pH<sub>ZPC</sub> = 8.75) more basic than that of double layer hydroxide (pH<sub>ZPC</sub> = 7.5), redox potential of LDH (-27 mV) was higher than that of Gh (-103mV), and the rH<sub>2</sub> of Gh (14.04) was higher compared to anionic clay (13.33).

**Keywords:** LDHs, Ghassoul, Redox Potential, Zero point of Charge, Antibacterial.

### 1. INTRODUCTION

The term of lamellar double hydroxides is used to designate synthetic or natural lamellar hydroxides with two kinds of metallic cations in the main layers and interlayer domains containing anionic species. This wide family of compounds is also referred to as anionic clays, by comparison with the more usual cationic clays whose interlamellar domains contain cationic species [1]. Their huge potential for anionic exchange [2], their oxidative-reducing or acid-base behaviour and their electrical properties [3, 4]; give them interesting properties opening the prospect of their application in many areas: catalysis, environment, food industry, pharmaceutical industry, polymer industry and solid electrochemistry [5, 6]. Ghassoul 'Gh' is a Moroccan natural cationic clay rich in magnesium, mostly live smectite. This clay has attracted the interest of many chemists since the last century [7] and is of significant industrial interest [8]. Anson and Oyama [9] showed that iron in cationic clays could intervene in redox

catalysis mechanisms. The use of clays also fascinated microbiologists, the work of Haydel et al., (2008) [10], has shown that several bacterial species (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*,...) are neutralized by different clays diluted at 500mg/ml. Indeed, important cellular processes are located at the level of the cell membrane, which is the reactive surface controlling chemical hosting, and can vary depending on the environment [11, 12]. As a result, the adaptation of bacteria to various environmental stresses depends on their ability to alter the chemistry of their surface [13]. As a matter of fact, there are three general mechanisms by which bacteria can host high concentrations of ions that can be toxic to the species: (i) ions can be expelled from the cell by efflux [14]; (ii) metal ions can be complicated into non-toxic molecules such as thiols in the surrounding solution, or (iii) metal ions can be reduced to a less toxic oxidation state in the cell [15].

While for metallic nanoparticles, there are three main lethal mechanisms: the formation of reactive

oxidizing species (ROS), the ion release process and, finally, the interaction of NPs with the cell membrane. Metal nanoparticles, compared to their salts, have an increased potential to fight bacterial infections [16, 17]. The size of the NPs mainly influences the antibacterial mechanism [18, 19]. Thus, nanoparticles and nanoparticle-based devices are of interest in numerous industrial applications due to their unique and often advantageous properties [20]. Indeed, Clay minerals have a different and interesting set of properties [21]. Moreover, to characterize the electron activity independently of the proton activity (pH), the chemical notion of rH<sub>2</sub> is used in various disciplines linked to living organisms, including biological physics [22, 23]. Like pH, rH<sub>2</sub> is defined as: (rH<sub>2</sub> = log [1/H<sub>2</sub>]), where [H<sub>2</sub>] is the thermodynamic activity of molecular hydrogen that would be formed following electron exchanges between water and solute species (Országh 1992). rH<sub>2</sub> is therefore a notion equivalent and complementary to pH [24]. Thus, the calculation of the value of rH<sub>2</sub> from the experimental measurement of the Eh potential and the pH allows evaluating the strength of an oxidant (or a reducer) by identifying the electron transfers alone [25]. The rH<sub>2</sub> value is therefore an equivalent and complementary concept to the pH [22].

Taking into account the numerous applications of Gh and LDH, this work aims to study if there is a correlation between their electrochemical characteristics (point of zero charge (pzc), redox potential, antioxidant power, chemical composition) and their antibacterial effect. Before these experiments, the materials were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM).

## 2. EXPERIMENTAL PROCEDURES

### 2.1. Materials

The cationic clay used is the Ghassoul coming from Jbel Ghassoul in the region of Fez-Meknes in Morocco, marketed under the name of (Ghassoul Chorfa Al Akhdar). The chemicals used to synthesize the lamellar double hydroxides: zinc chloride (ZnCl<sub>2</sub>, LOBAChemie, 97%), aluminum chloride (AlCl<sub>3</sub>, 6H<sub>2</sub>O, LOBAChemie., 98%), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, LOBAChemie, 98%) and sodium

hydroxide (NaOH, LOBAChemie, 98%) were used without further purification. Deionized water was used as a solvent throughout this study.

### 2.2. Preparation of Samples

The Ghassoul was crushed without any treatment. After drying it at 100°C, it was ground and sieved with standardized sieves according to AFNOR with a diameter between 250 and 315 μm [26]. The double lamellar hydroxide prepared by the co-precipitation method. This method consists of preparing an aqueous solution containing a mixture of NaOH and Na<sub>2</sub>CO<sub>3</sub> and another solution containing a mixture of salts AlCl<sub>3</sub> and ZnCl<sub>2</sub> with a molar ratio Zn/Al=3. The precipitation is caused by adding the two solutions drop by drop in a flask containing 20 ml of distilled water to maintain a pH = 10. Then, the mixture was stirred at 70°C for 18 h until crystallization. The precipitated product was filtered and washed twice in the filter with warm water to remove excess ions (Cl<sup>-</sup>, Na<sup>+</sup> ...), followed by drying at 105°C for 18 h [27, 28]. The products were characterized using XRD, FTIR, and SEM-EDX

### 2.3. Fourier transform infrared spectroscopy (FTIR)

The infrared analysis was performed using Fourier Transform Infrared Spectroscopy (FTIR) with a JASCO 4000 spectrometer, equipped with a detector (TGS) and a ceramic source. The absorption spectra were performed with tablets containing 1 mg sample and 100 mg KBr, and recorded in the range 4000 - 400 cm<sup>-1</sup>, with a resolution of 4 cm<sup>-1</sup>.

### 2.4. XRD

The XRD analysis was performed by the powder method using a Philips PW 1800 diffractometer (copperK $\alpha$  line  $\lambda$  = 1.5406 Å, 40 kV, 20 mA) in Bragg- Brentano geometry. The spectra of the prepared materials were recorded in a range of 2 $\theta$  equal 5° - 70° with an angular step of 0.04°.

### 2.5. SEM and EDX analysis

The SEM (Scanning Electron Microscopy) and EDX (Energy dispersive X-Ray analysis) were utilized to determine the morphology and elemental composition of the Gh and LDH.

### 2.6. Determining the zero point of charge (zpc)

The pH of the zero charge point (pH<sub>zpc</sub>) is the pH

value for which the net load of the surface of the adsorbent is zero [29]. The  $pH_{zpc}$  was determined by putting 50 ml of a NaCl solution (0.01 M) in six closed vials, and then their pH is adjusted to values of 2, 4, 6, 8, 10 and 12, by the addition of NaOH or HCl (0.1 M). Fifty mg of the material's sample is then added to each vial. The suspensions were kept agitated at room temperature for 24 hours. The final values of pH ( $pH_f$ ) were subsequently raised. The value of  $pH_{zpc}$  is the point at which the curve of pH ( $pH_f - pH_i$ ) according to the  $pH_i$  crosses the line equal to zero [29, 30].

## 2.7. Anti-oxdyant activity

### 2.7.1. DPPH radical scavenging activity assay

The chemical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was one of the first free radicals used to study the structure-antioxidant activity relationship of phenolic compounds; it has an unpaired electron on a bridge atom, nitrogen [31]. The reduction of the free radical DPPH by an antioxidant can be followed by UV-Visible spectrometry, by measuring the decrease in absorbance at 517 nm caused by the antioxidants [32]. The scavenging effects of samples for DPPH radical were monitored according to the method of Shimada et al., [33]; Yen [34] and Qin et al., [35]. All measurements were done in triplicate. The percentage (%) inhibition activity was calculated from the following equation 1:

$$\% \text{ Inhibition DPPH} = (A1 - A2) / A1 \times 100 \quad [36]$$

Where, A1 is the absorbance of the control (containing all reagents except the test compound), and A2 is the absorbance of the test compound.

The IC<sub>50</sub>, the efficient concentration of compound decreasing the initial concentration of DPPH radical by 50% was obtained by interpolation from regression analysis.

The same procedure was used for standard antioxidant compounds, namely acid ascorbic.

### 2.7.2. Ferric Reducing Power (FRAP) Assay

The reducing power of iron ( $Fe^{3+}$ ) in the clay is determined according to the method described by Yen and Chen [34] and Boucherit-Otmani et al. [37]. All measurements were done in triplicate. An increase in absorbance corresponds to an increase the reducing power of the extracts tested.

## 2.8. The physicochemical aspect of the bioelectronic coordinates (pH, rH2 and Eh)

Five g of clay are suspended in 100 ml of distilled water. The suspension was stirred for one hour to achieve balance between clay and water [38]. After standing the measurements were carried out for the pH and Eh. The tests were carried out under the same conditions for the two materials tested, with three repetitions. The potential Eh (expressed in mVolts) and the pH are measured using conventional electrodes (with pH meter Adwa AD1000). The rH2 value measures the amount of electrons available that can be exchanged in the aqueous phase. As the classic redox potential Eh incorporates the proton content into its value, it is essential to use the rH2 unit, which is independent of it [39].

The redox conditions are also dependent on the pH, in accordance with the following formula:

$$rH2 \text{ (at } 25^\circ\text{C)} = 33.3 \text{ Eh (volts)} + 2pH \quad [24]$$

## 2.9. Antibacterial tests

### 2.9.1. Preparation of clays

The solutions studied at 500 mg/ml concentrations, were prepared in suspension in the distilled water and mechanically agitated by vortex, then sterilized in an autoclave at 121°C for 20 minutes.

### 2.9.2. Preparation of inoculums

Cultures of the bacteria were grown on nutrient agar (Mueller-Hinton) for 18 to 24 hours and incubated at 37°C. Then, these cultures were suspended in saline solution (0.9% NaCl) and inoculated respecting a density equivalent to McFarland standard density 0.5 or an absorbance included between 0.08 and 0.13 at 625 nm, which corresponds to an inoculums of approximately 1 to 210<sup>8</sup> CFU/ml [40]. The concentration of the inoculums used for the antibacterial test was 5 10<sup>5</sup> CFU/ml.

### 2.9.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined according to the technique of the microtiter on microplate described by Eloff and Andrews [41, 42]. The MTT 2-(4, 5-dimethyl-2-thiazolyl) -3, 5-diphenyl -2H-tetrazolium bromide was used as an indicator of bacterial viability. 50 µl of Muller-Hinton broth were added

to each well of a microtiter plate containing 96 wells, and 50 $\mu$ l of the stock aqueous suspension of clays (500 mg/ml) were added to the first well of each row from which a series of geometric dilution common ratio of two were made.

#### 2.9.4. Determination of minimum bactericidal concentration (MBC)

Referring to the results of the MIC assay, the wells showing complete absence of bacterial growth were identified, and 10  $\mu$ l of each well were transferred to Mueller-Hinton agar plates and incubated at 37°C for 24 h. The complete absence of growth was considered as the minimum bactericidal concentration [43]. Effect was determined according to the value of the CMB / MIC ratio. When the CMB/MIC ratio is greater than or equal to 4, the material tested is considered to be a bacteriostatic agent, if on the other hand it is less than 4, the material tested is considered to have a bactericidal effect [44].

#### 2.10. Statistical analysis

The data were presented as means, and the statistical analyses were performed using Microsoft Office Excel and OriginPro 8.

### 3. RESULTS

#### 3.1. Physicochemical Characterization of the clays tested

##### 3.1.1. XRD diffractogram of the Zn<sub>3</sub>Al-CO<sub>3</sub>

The XRD diffractogram of the Zn<sub>3</sub>Al-CO<sub>3</sub> obtained (Fig 1) shows the formation of a well-crystallized phase characteristic of the LDH structure [45]. The main peaks corresponding to the basal planes (003) and (006) are present at low values of 2 $\theta$  equal to 11.40° and 23.08° respectively [28]. They are characteristic of the lamellar structure of the synthesized sample [46, 47].

##### 3.1.2. XRD diffractogram of the Ghassoul

The XRD spectrum (Fig 2) of Ghassoul, is similar to that obtained by Allaoui et al., [48]. It shows that the dominant phase is stevensite (s) with the presence of quartz (Q) and dolomite (D). XRD analyses showed that Gh consists of three phases of clay [49]:

- Stevensite (S) observed at the 2 $\theta$  = 5.44°, 11.56°, 19.52°, 29.02°, and 44,84°;

- Dolomite (D) observed at the 2 $\theta$  = 30.78°, 35.9°, 40.98°, and 43.26°;
- Quartz (Q) observed at the 2 $\theta$  = 20.64°, 26.58°, and 51.30° characteristics of the quartz phase

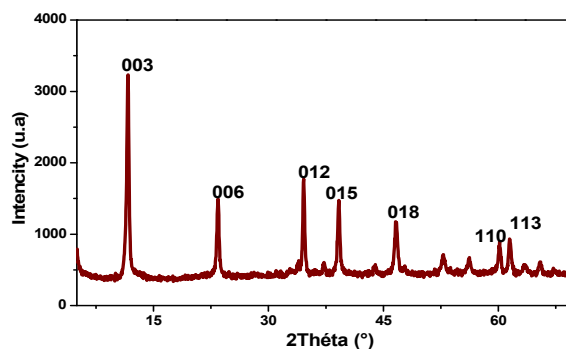


Fig. 1. Diffractogram of LDH-Zn<sub>3</sub>Al-CO<sub>3</sub>

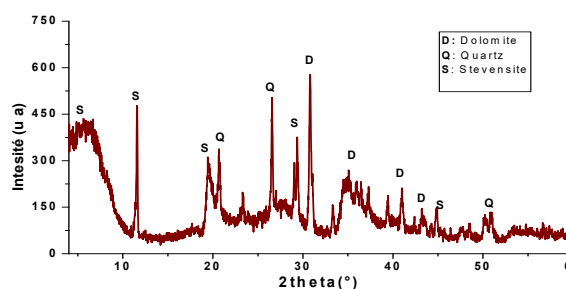


Fig. 2. Diffractogram of Ghassoul

#### 3.1.3. Infrared spectroscopy analyses

The spectrum FTIR of Ghassoul (Fig 3) demonstrates a large absorption band at 3419 cm<sup>-1</sup> corresponding to the OH-stretching vibration of the water molecules. The bending mode of the interlayer and/or adsorbed water appears at 1637 cm<sup>-1</sup>. The absorption band at 1450 cm<sup>-1</sup> is characteristic of CO stretching of carbonates due to the presence of calcite in Gh. The thin and intense band at 1017 cm<sup>-1</sup> corresponds to the elongation vibration of the Si-O-Si silica. Both vibrations of ( $\nu_s(\text{CO}_3^{2-})$ ) and Al<sub>2</sub>(OH)<sub>6</sub> groups are observed at 880 cm<sup>-1</sup>. The bands which appear around 666 and 462 cm<sup>-1</sup> are attributed to the vibrations of the Si-O-Mg and Si-O-Si respectively [50, 51]. The absorption band at 666 cm<sup>-1</sup> is exhibited only in the case of stevensite in the smectite group [52]. The absorption band at 794/796 cm<sup>-1</sup>, related to the angular deformation of the Fe-OH and Mg-OH bonds [53].

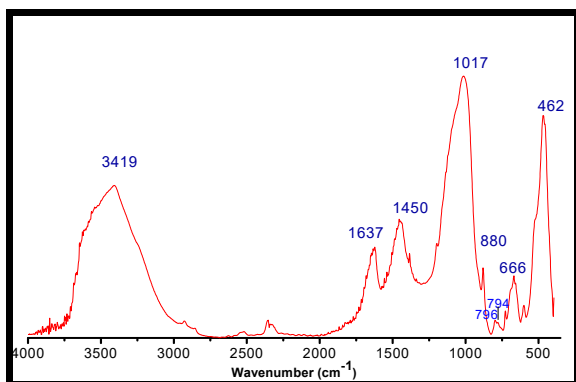


Fig. 3. FTIR spectra of Ghassoul

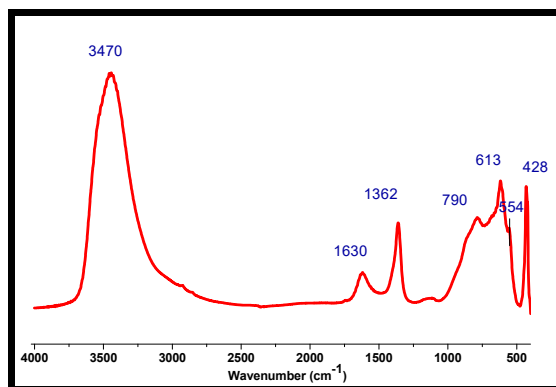


Fig. 4. FTIR spectra of Zn<sub>3</sub>Al-CO<sub>3</sub>

**3.1.4. SEM /EDX Analysis of Ghassoul and LDH-Zn<sub>3</sub>Al-CO<sub>3</sub>**

The SEM micrograph of Gh (Fig 5) shows that the particle morphology has an automorphic petalloid-like microstructure, typical of that of smectite clay [56, 57].

EDX analysis of Ghassoul (Fig 6, Table 1) show that clay has a higher percentage of Oxygen (47, 9%) and Silica (23, 8%) The other elements Mg, Ca, Fe, Al, S and k presented 13.8, 8.2, 4, 1, 0.7

and 0.6% respectively. These results are in agreement with XRD.

The SEM image (Fig 7) shows that LDH is in the form of parallel sheets and platelet morphology, in agreement with the typical structure of lamellar double hydroxides.

EDX model (LDH) and Table 2 shows that the intensity of the Zn peak is the highest with a weight percentage of 55.77. The other elements O, Cl, Al and C presented a percentage of 24.84, 10.08, 6.39 and 2.92% respectively.

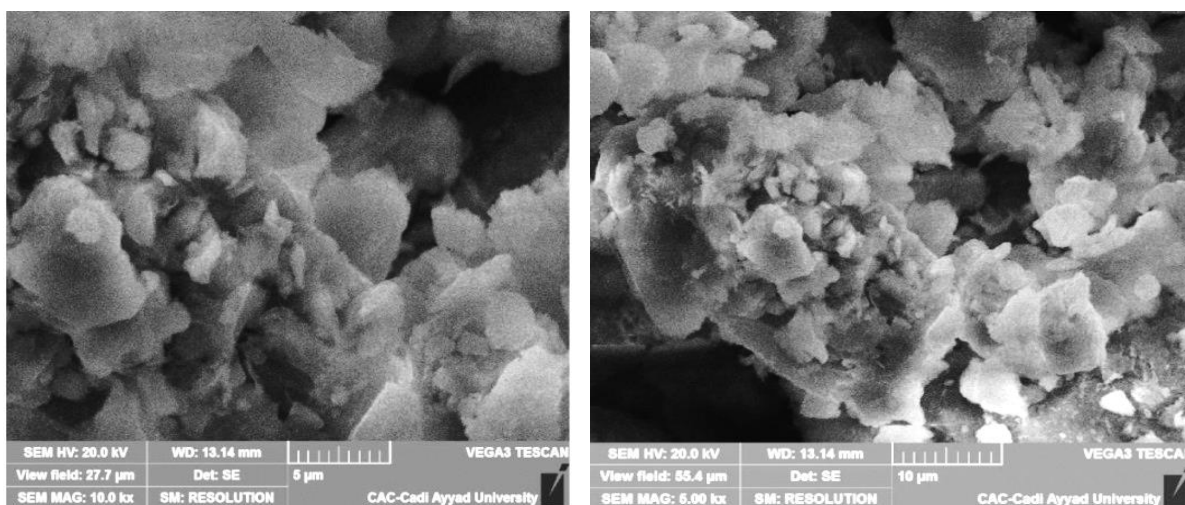


Fig. 5. SEM micrograph of Ghassoul.

Table 1. The percentage (%) of Chemical elements of Ghassoul

Element	O	Fe	Mg	Al	Si	S	K	Ca
Weight %	47,9	4	13,8	1	23,8	0,7	0,6	8,2

Table 2. The percentage (%) of Chemical elements of LDH

Element	C	O	Al	Cl	Zn	Total
Weight %	2.92	24.84	6.39	10.08	55.77	100

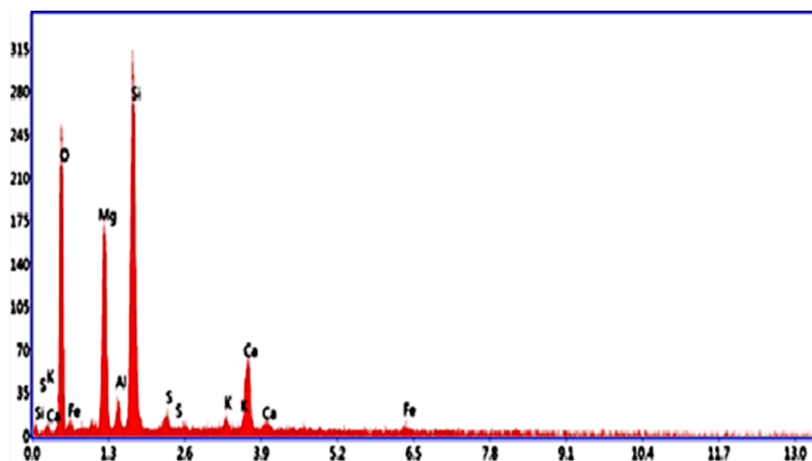


Fig. 6. EDX analysis of Ghassoul

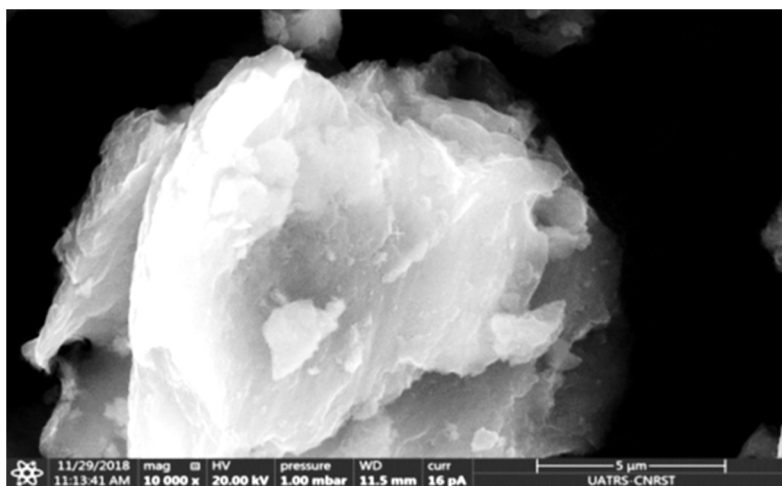


Fig. 7. SEM images analysis of LDH

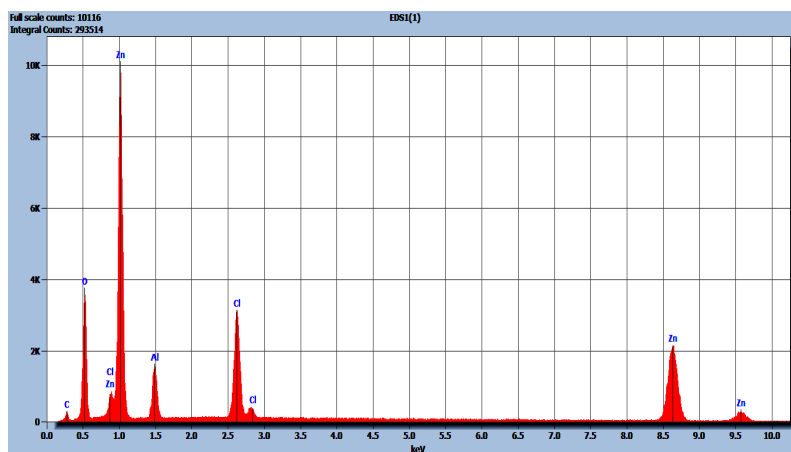


Fig. 8. EDX analysis of LDH

### 3.2. Zero point of charge

The results of the determination of the zero point of charge (zpc) of the two clays (Fig 9) showed that the Ghassoul had a basic  $pH_{zpc}$  equal to 8.41,

while that of the LDH was more or less neutral of the order of 7.5. Mekdad et al. [58], have obtained the value of 7.94, 7.97 and 7.67 for Hydrotalcite Mg-Al with Mg/Al ratio equal to three

respectively by the method of potentiometric titration with the electrolyte NaCl, conductometric titration and pH derivative (method used in this work), a value similar to that of our LDH material. For Ghassoul, Moussout et al. [57] had found, by potentiometric and the conductometric titrations with different electrolytes (NaCl, CsCl, NaF, NaBr et LiCl), that the zero point of charge is located at  $\text{pH} = 10.7$ , which is Higher value than that obtained in this work by the pH drift method. This difference can be attributed to the method used in the determination of the zpc.

The range of  $\Delta\text{pH} > 0$  ( $\text{pH}_f > \text{pH}_i$ ) (Fig 9) correspond to the release of  $\text{OH}^-$  and  $\text{CO}_3^{2-}$  ions for LDH, in addition to basic oxides in the case for the Ghassoul. The domain where  $\Delta\text{pH} < 0$  ( $\text{pH}_f < \text{pH}_i$ ) is explained by an excess of negative charges not neutralized by the release of the protons ( $\text{H}^+$ ) [30]. On the acid side of the zpc, the system is positively charged and on its alkaline side it is negatively charged [59].

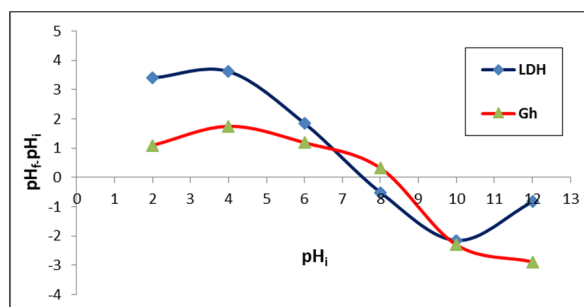


Fig. 9.  $\text{pH}_{\text{zpc}}$  of the clays tested

Indeed, when the pH of the solution is lower than  $\text{pH}_{\text{zpc}}$ , the functional groups on the surface of the material will be protonated by the excess  $\text{H}^+$  ions in the solution then the surface of the clay will be positively charged. On the other hand, if the pH of the solution is higher than that of  $\text{pH}_{\text{zpc}}$ , the functional groups on the surface of the adsorbent will be deprotonated by the  $\text{OH}^-$  ions present in the solution and the surface of the clay will be negatively charged [60, 61].

Therefore, the charge of the Gh surface will be positive when the solution  $\text{pH} < 8.41$ ; negative when  $\text{pH} > 8.41$  and neutral when  $\text{pH} = 8.41$ . While the surface charge of LDH will be positive at solution  $\text{pH} < 7.5$ ; negative at solution  $\text{pH} > 7.5$  and neutral at solution  $\text{pH} = 7.5$ . This reveals that the zone where the surface of the material is positively charged is wider for the Ghassoul than

for the hydrotalcite tested.

### 3.3. Antioxidant activity

#### 3.3.1. DPPH radical scavenging activity assay

Fig. 10 shows that the inhibitory capacity of DPPH radicals is proportional to the concentrations of the different materials tested. In the other hand, the reduction of DPPH is accompanied by its passage from the violet color characteristic of DPPH solution to the yellow color measurable at 517 nm. In fact, the capacity for scavenging hydroxyl radicals increased from 1% to 31% for LDH and from 2% to 57% for Gh, respectively for concentrations which also increased from 0.31 mg/ml to 10 mg/ml.

Ghassoul therefore exhibits good activity for inhibiting DPPH radicals compared to LHD material. The percentage of DPPH inhibition by ascorbic acid (vitamin C: vitC) is greater than those recorded for the two materials tested at different concentrations. Regression analysis of the graphs showed that  $\text{IC}_{50(\text{LDH})} = 16.97$  mg/ml,  $\text{IC}_{50(\text{Gh})} = 8.09$  mg/ml and  $\text{IC}_{50(\text{vitC})} = 0.34$  mg/ml. These results enabled us to classify their anti-radical activity as follows: Ascorbic acid > Ghassoul > LDH.

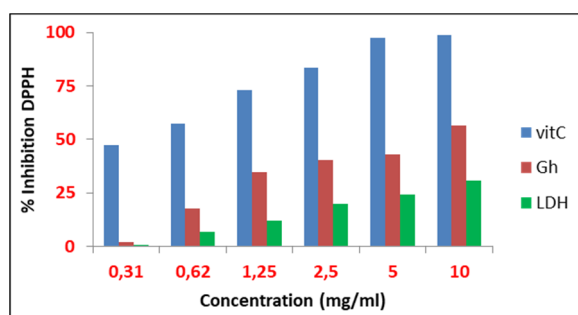


Fig. 10. The percentage (%) inhibition activity of DPPH

#### 3.3.2. Ferric Reducing Power (FRAP) Assay

Fig. 11 shows that the reducing power of iron III varies linearly with the concentrations of the two materials (Ghassoul, LDH). For the maximum concentration used 10 mg/mL, Gh solution showed an absorbance of 0.27, and the HDL solution showed an absorbance of 0.19. The ascorbic acid used as a reference shows a reducing power greater than the other two clays. This test is consistent with the DPPH test in which ascorbic acid > Ghassoul > HDL. Based on these results, it is possible to deduce that the cationic

clay studied has a radical scavenging capacity and a greater reducing power than anionic clay (LDH).

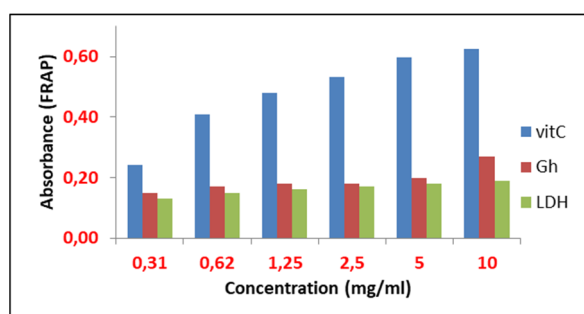


Fig. 11. Ferric Reducing Power (FRAP)

### 3.4. The clays tested and the physico-chemical aspect of the bioelectronic coordinates (pH, rH2, and Eh)

The results obtained (Table 3) showed that the LDH had an Eh of -27 mV, and the measurement of Eh for the Ghassoul was -103 mV. This showed that Ghassoul is more reducing than LDH, which is in agreement with the results of the anti-oxidant test (paragraph 3.3).

Moreover, the calculation of rH2 from the experimental measurement of the Eh potential and pH, allowed us to evaluate the oxidizing or reducing force of the two clays, by identifying only the transfers of electrons [25, 62]. Indeed, the Gh displayed an rH2 of 14.04, while that of LDH was 13.33. This shows that the Gh has a greater oxidizing force than anionic clay.

Table 3. Eh, rH2 and pH of the clays tested

	pH	Eh (volts)	rH2
Gh	8,75	-0,103	14,04
LDH	7,12	-0,027	13,33

### 3.5. Antibacterial test

The results obtained (Table 4) show that the LDH has a MIC of 6.2 mg/ml for *Enterococcus faecalis* (EF) and *Staphylococcus aureus* (*S. aureus*), while for *Escherichia coli* (*E. coli*) it is lower, of the order of 4.3 mg/ml. Ghassoul showed a MIC

of 4.3 mg / ml for the two Gram-positive bacteria *E.F* and *S. aureus*, while for *E. coli*, the MIC is lower; it is 2.1 mg/ml. We deduce from these results that the Gh presents lower MICs of approximately 50% compared to those obtained with  $Zn_3Al-CO_3$ , an efficiency twice as high for Gassoul.

It should also be noted that the bactericidal effect of the two materials tested was detected only in the Gram-negative tested bacteria (*E. coli*), but bacteriostatic effect in the Gram-positive tested bacteria (Table 4). In addition, the bacteria tested exhibit multi-resistance to someone antibiotics used in therapeutic treatment of the resulting infections, which suggests a possible opportunity for the fight against multi-resistance to antibiotics. This bactericidal power observed in this study also indicates a significant eradication capacity of fecal germs, of which *Escherichia coli* is the main representative.

Cationic clay has CMB of 5.17 mg/ml for (*E. coli*) and a CMB/MIC ratio of less than 2, which gives it a very important bactericidal activity on Gram-negative and a growth inhibitory effect on Gram positive bacteria. That is, an antibacterial power with a broad spectrum.

## 4. DISCUSSION

It is important to realize that the effectiveness of the antimicrobial is largely dependent on the targeted bacterium. An antimicrobial agent that is bactericidal for one particular bacterial species may be bacteriostatic for another.

Moreover, various antibacterial agents vary considerably in their range of effectiveness [63]. Indeed, the bacterial cell wall has an amphoteric property, due to the presence of cationic and anionic groups [64]. In Gram-positive bacteria, the reason for the negative charge is the presence of teichoic acids linked to either the peptidoglycan or to the underlying plasma membrane. These teichoic acids are negatively charged because of the presence of phosphate in their structure.

Table 4. MIC and MBC of the clays tested

	MIC (mg/ml)			MBC (mg/ml)			MBC/MIC
	<i>S. aureus</i>	<i>E. F</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. F</i>	<i>E. coli</i>	<i>E. coli</i>
LDH	6,2	6,2	4,13	-	-	6,2	1,5
Gh	4,13	4,13	2,1	-	-	4,13	1,9



The Gram-negative bacteria have an outer covering of phospholipids and lipopolysaccharides. The lipopolysaccharides impart a strongly negative charge to the surface of Gram-negative bacteria cells [64]. In addition, the pH dramatically affects bacterial growth. Each species develops in a defined pH range and has an optimum pH of growth, neutrophil bacteria have an optimum pH between 5.5 and 8 [65], case of bacteria tested. Moreover, according to Kimbrough's hypothesis, bacteria have an optimum oxidation-reduction potential (ORP) for their growth. Consequently, the more their environment has an ORP far from this optimum, the more their growth is suppressed [66]. Each type of microorganism is adapted to specific Eh conditions and is characterized by its ability to develop in a more or less wide Eh range. For example, anaerobic bacteria can only grow within a narrow range of very low Eh values. Aerobic microorganisms require a higher Eh [67, 68]. In the work described by Kimbrough et al (2006), bacterial growth is directly correlated with changes in Eh [66]. We can therefore assume that the difference in MIC between the two clays can be attributed to their redox potential, which is due to the difference in their chemical composition, which has an impact on their pH and rH<sub>2</sub>. This can be explained by the pH<sub>Zpc</sub> (8.41) of Ghassoul which is more basic than that of the hydrotalcite tested (pH<sub>Zpc</sub> = 7.5), by ORP of LDH (-27 mV) which was higher than that of Gh (-103 mV), and by the significant oxidizing power (rH<sub>2</sub>) of Gh compared to anionic clay. Furthermore, the possibility exists that the antibacterial activity could be attributed to the chemical composition (Table 1 and 2) of the clays, since ghassoul is composed of reducing elements (Mg, Fe, Ca, K). Whereas LDH contains zinc and aluminum, which are amphoteric chemical elements; their activity depends on the pH of the medium. Moreover, the importance of the bactericidal effect of ghassoul compared to LDH can be explained by the presence of iron. Indeed, the Fenton reactions lead to the oxidation of Fe<sup>2+</sup> bound to minerals to generate hydroxyl radicals that can damage cells [69, 70].

## 5. CONCLUSIONS

In this work, a layered double hydroxide Zn<sub>3</sub>Al-CO<sub>3</sub> (anionic clay) was successfully

synthesized. This compound and the Ghassoul (cationic natural clay) were characterized by different techniques (DRX, IRTF, SEM, EDX). The two materials presented a good structure and a good purity.

Thus, we can say that the majority presence of basic reducing elements was responsible for the lower redox potential and an increase in rH<sub>2</sub> for ghassoul, which adversely affected the growth of bacteria tested.

In summary, the importance of this study relies on its originality. This is the first attempt to compare the antioxidant activity and antimicrobial effect of anionic clay of the Hydrotalcitic type and cationic clay the Ghassoul, to contribute to the depollution and improvement of water quality in the environment and to the fight against bacterial resistance to antibiotics.

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