

# Study On Antibacterial Activity Of Cu/Al Layered Double Hydroxide In Aqueous Solution

A.ARIUNJARGAL<sup>1</sup>, TS. RENTSSENKHAND<sup>2</sup>, UNG SU CHOI<sup>3</sup>, D.KHASBAATAR<sup>1†</sup>

<sup>1</sup>NATIONAL UNIVERSITY OF MONGOLIA, SCHOOL OF ENGINEERING AND APPLIED SCIENCES

<sup>2</sup>LABORATORY OF MICROBIAL SYNTHESIS, INSTITUTE OF GENERAL AND EXPERIMENTAL BIOLOGY, MONGOLIAN ACADEMY OF SCIENCES. MONGOLIA

<sup>3</sup>NATIONAL AGENDA RESEARCH DIVISION, KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY, REPUBLIC OF KOREA.

## Abstract

In this study, a novel Cu/Al layered double hydroxide (Cu/Al-LDH) was synthesized using the co-precipitation method. Characterizations of the Cu/Al-LDH were well determined with an X-ray. The results showed that the antibacterial activity at the Cu/Al-LDH concentration was 100 mg/ml, with the highest bacterial growth inhibition. The clear range, which inhibited the growth of Gram-positive, *Bacillus subtilis* bacteria, was 8 mm. The antibacterial activity depends on the concentration of Cu/Al-LDH, and the size of the clear experimental range shows that the antibacterial activity of *B.sub* is high over time. Cu/Al-LDH material is active against Gram-negative and positive bacteria, pathogens, and household contaminants so that it can be used for further disinfection.

## Introduction

Many types of bacteria and microorganisms can cause disease in humans and living organisms, and various drugs have been developed and used in practice. [1]. Nowadays, antibacterial agents with metal ions such as zinc, copper, nickel, cobalt, and manganese have been producing several types of inorganic materials [2-4]. These antibacterial materials contain metal ions. At the same time, it is hypothesized that a highly positively charged surface interacts with a negatively charged surface of the bacterium, thereby inhibiting bacterial activity [5]. Therefore, positively charged materials containing metals may be suitable for antibacterial use. LDH is relatively inexpensive and easy to obtain compared to antibacterial materials containing metals such as Au, Ag, and TiO<sub>2</sub> [5-7]. There are many ways in which LDH can be synthesized into layered hydroxides [8-9]. The most widely used method is co-precipitation. This method uses an aqueous solution containing M<sup>2+</sup>, M<sup>3+</sup> cations, and anions to synthesize the LDH [10]. This study demonstrates the acquisition of effective antibacterial properties of Al-based LDHs with Cu. Accordingly, this study can efficiently synthesize LDH and determine antibacterial activity by disk proliferation experiment. In addition, this work is aimed at improving the understanding of LDH-based antibacterial agents.

## Methods And Materials

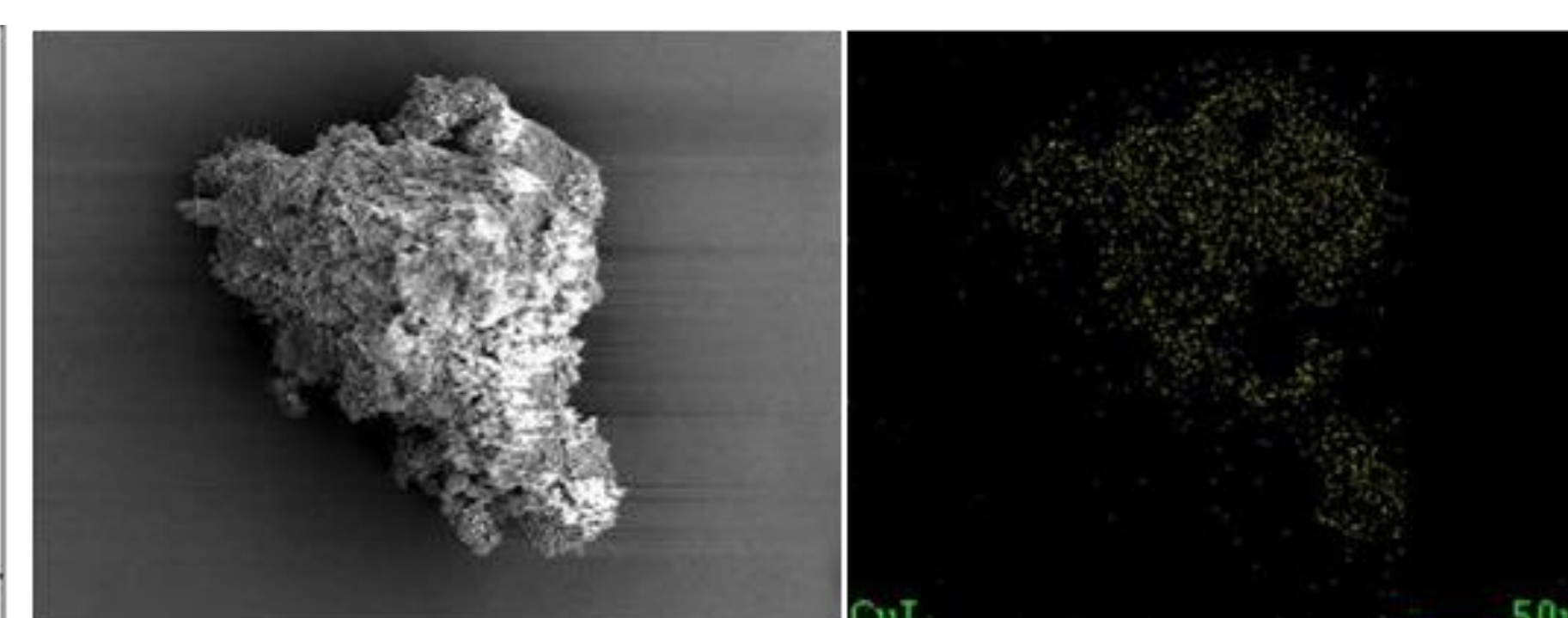
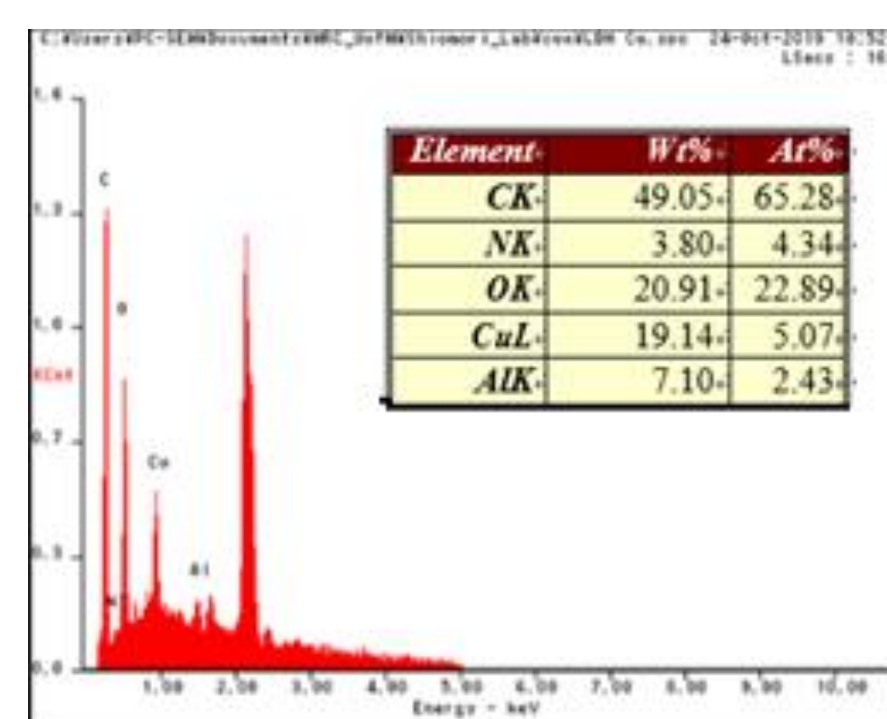
Copper chloride dehydrate, aluminum chloride hexahydrate, sodium carbonate, and sodium hydroxide (CuCl<sub>2</sub>·2H<sub>2</sub>O; AlCl<sub>3</sub>·6H<sub>2</sub>O; Na<sub>2</sub>CO<sub>3</sub>; NaOH Fluka Chemical Co.; ≥99 pure), were used without further purification. The bacterium strains used in this work are *Bacillus subtilis* (B.sub).

### Synthesis of Cu/Al-LDH

Cu/Al-LDH material was synthesized using traditional co-precipitation method. Technically, 24.1 g of MgCl<sub>2</sub>·6H<sub>2</sub>O (0.141 M) and 9.6 g of AlCl<sub>3</sub>·6H<sub>2</sub>O (0.0398 M) were dissolved in 600 cm<sup>3</sup> distilled water (Solution 1). Solution 2 was prepared by dissolving 31.8 g of Na<sub>2</sub>CO<sub>3</sub> (0.3 M) in 400 cm<sup>3</sup> distilled water. Solution 1 and 2 was added into 1 L distilled water under constant stirring at a temperature of 25°C (at ambient temperature), then the pH of the mixed solution was kept at 10 by adding 1 M NaOH. The mixture was aged at 60°C for 4 h. Then the precipitate was separated by a filter paper and washed with distilled water. The wet solid was dried at 50°C for 24 h to obtain the MgAl-LDH [10].

### Antibacterial studies

Two different nutrient media were prepared for *B.sub* and *E.coli* bacteria. Nutrient broth (NB) is the culture medium for *B.sub* culture. Lauria-Bertani (LB) is the culture medium for *E.coli*. NB and LB were prepared in 100 ml of medium, respectively. LB: Tryptone - 1 g, Yeast extract - 0.5 g, NaCl - 1 g, NB: Beef extract - 0.4 g, Peptone - 0.4 g, Glucose - 1 g, Yeast extract - 0.1 g were added and distilled water was added up to 100 ml [11].



## Results

### 3.1. Characterizations of X-Ray and SEM-EDX

Cu/Al-LDH was obtained 17.6 g of very fine powder using the co-precipitation method.

24.1 g (CuCl<sub>2</sub> × 2H<sub>2</sub>O) + 9.6 g (AlCl<sub>3</sub> × 6H<sub>2</sub>O) + 31.8 g (Na<sub>2</sub>CO<sub>3</sub>) = 17.6 g (Mg<sub>2.5</sub> Al (OH) 16 (CO<sub>3</sub>) 4 × H<sub>2</sub>O). The resulting Cu/Al LDH yield was 42.5%.

The primary indicator for the determination of LDH is the result of X-ray diffractometer (XRD) analysis, which is determined by the fact that the metals in the mineral structure form a layered double hydroxide. As shown from Figure 1, the values of 2θ in 10-16°, 22-25°, and 33-36° determine the essential characteristics of the LDH. Thus, the XRD pattern of hydrotalcite-like compounds shows such features [12].

The morphology and composition of Cu/Al-LDH were determined using SEM-EDX. The main parameter that determines the properties of LDH is the molar ratio of the metals. In the SEM-EDX analysis of the Cu/Al-LDH, the ratio of metals is the same as that of the metals originally in solution.

The SEM-EDX study shows that the ratio of metals in Cu / Al-LDH is 2.7: 1, which corresponds to the ratio of metals in the original solution. SEM and element mapping analysis of Cu/Al-LDH, Figures 2.2 and 2.3, show that copper is evenly distributed in Cu/Al-LDH by elemental mapping analysis. From the above analysis, it can be seen that Cu / Al LDH obtained by co-precipitation method is a high purity material.

Table 1. Label in 24pt Calibri.

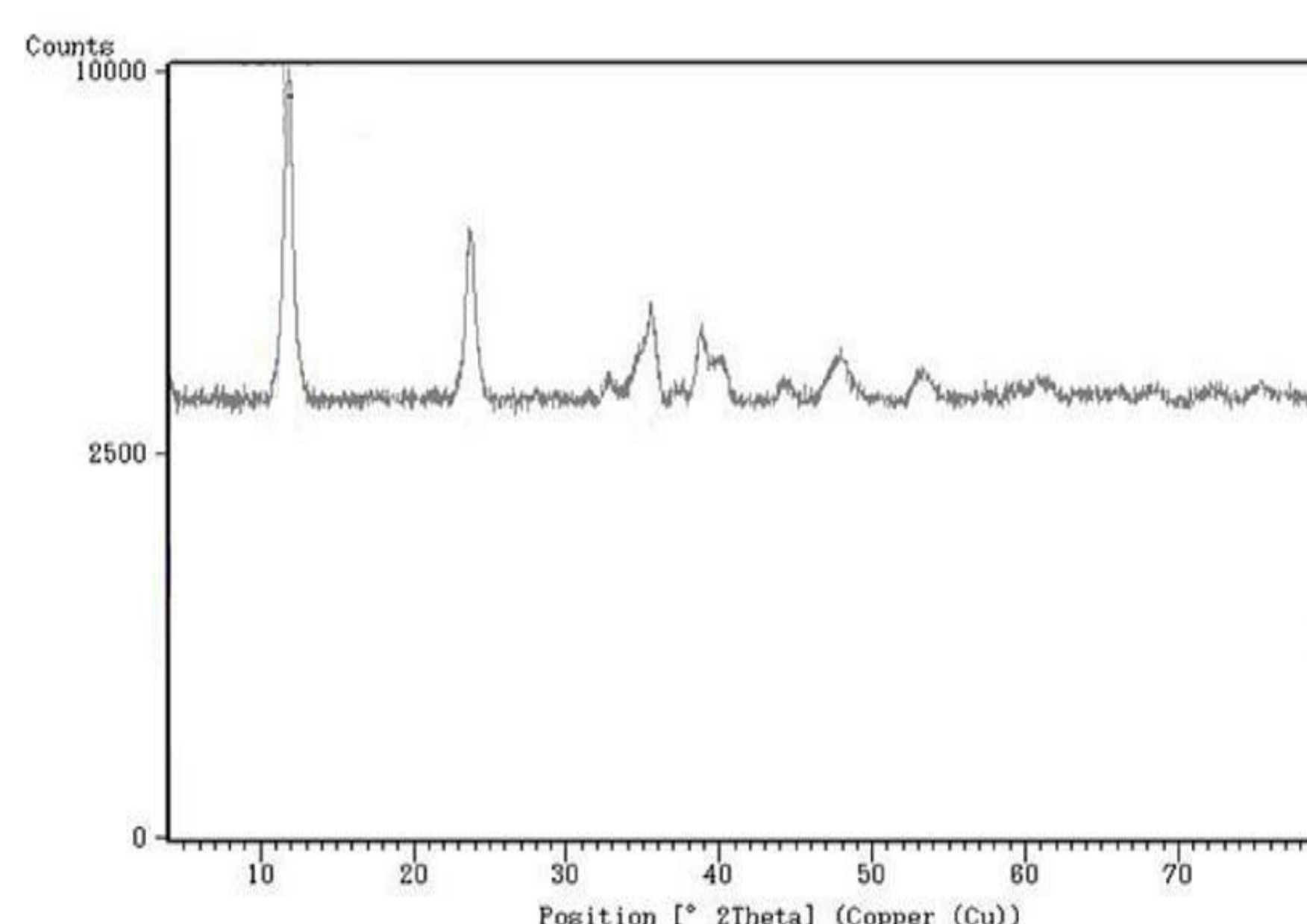
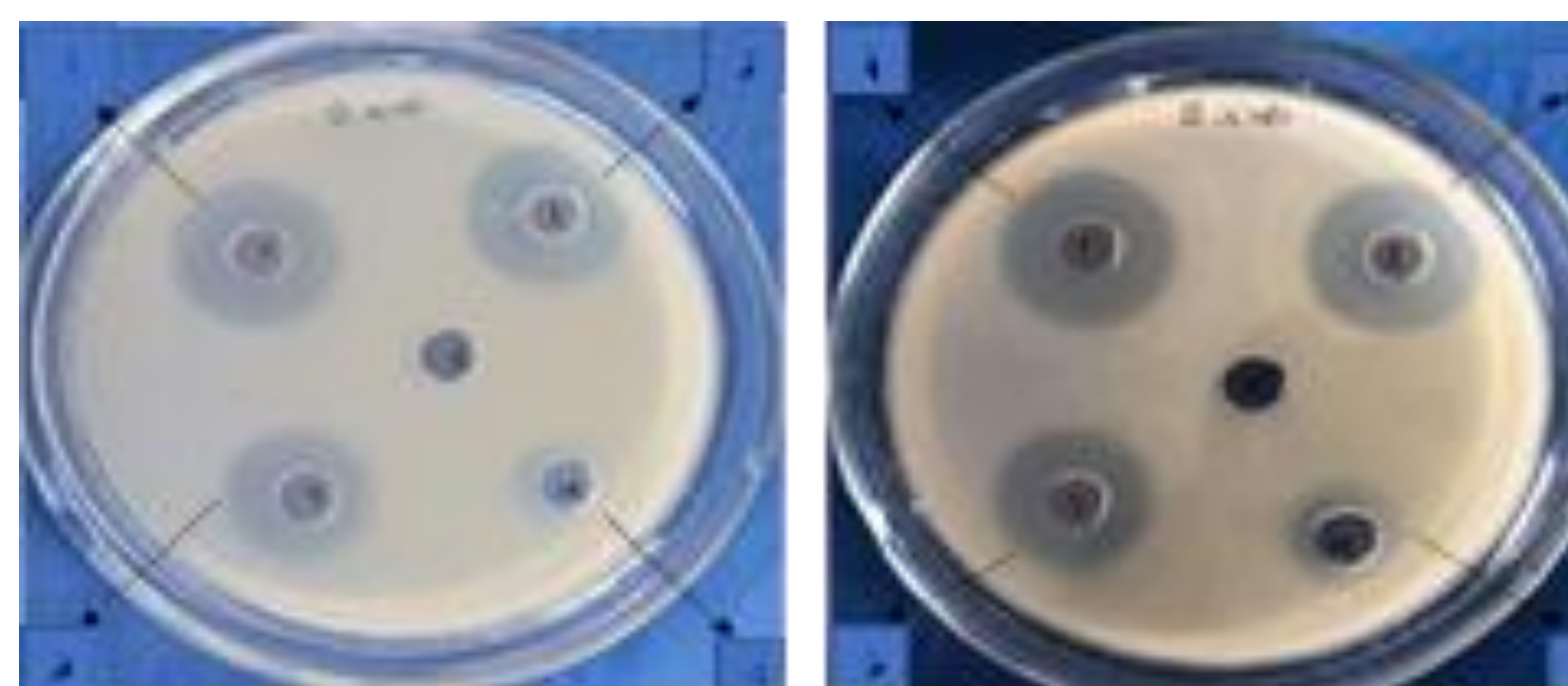


Chart 1. Label in 24pt Calibri.



## Discussion

Cu/Al-LDH solution with *B.sub* bacteria in 4 different concentrations: At 48 hours of antibacterial activity of Cu/Al-LDH were at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, the range of growth inhibition were 8 mm, 7 mm, 5 mm, and 3 mm, respectively. At 24 hours, 7 mm at 100 mg/ml, 6 mm at 50 mg/ml, 4.5 mm at 25 mg/ml, and 2 mm at 12.5 mg/ml were also presented for antibacterial activities of Cu/Al-LDH. For gram-positive *B.sub* bacteria, the 5-atom alcohols, glycerol, and phosphate (PO<sub>4</sub><sup>-</sup>), taichonic acid is found outside the cell wall [13]. Figure 3. *B.Sub* Antibacterial effect of Cu/Al-LDH. There are two reasons why Cu/Al-LDH is antibacterial for Gram-positive *B.sub* bacteria.

## Conclusions

The Cu/Al-LDH obtained by co-precipitation was determined using X-ray X-ray diffraction (XRD), Scanning electron microscopy and light spectrometer (SEM-EDX) spectroscopy, and element mapping.

From the above three types of analysis, Cu/Al-LDH obtained by co-precipitation was well synthesized. The antibacterial activity of the Cu/Al-LDH was observed in *B.sub* bacteria. The most significant inhibition of bacterial growth was observed at 48 hours when the Cu/Al-LDH was 100 mg/ml.

The antibacterial activity depends on the concentration of Cu/Al-LDH produced. The size of the translucent range of experiments shows that the antibacterial activity of *B.sub* is high over time.

## Future Directions

Our Cu/Al-LDH material is active against Gram-negative and positive bacteria that cause pathogens and household contaminants, which can be used for cleaning and disinfection.

## Contact Information

D. Khasbaatar  
National University of Mongolia  
Email: d\_khasbaatar@seas.num.edu.mn

## References

- B.Davaadorj "Microbiology", Ulaanbaatar, 2013.
- Hayhurst, Chris. "E. coli". The Rosen Publishing Group, 2004.
- Chih-Shan Li, Mei-Ling Hao, Wen-Hai Lin, Ching-Wen Chang, and Chiu-Sen Wang. *Evaluation of Microbial Samplers for Bacterial Microorganisms*. Aerosol Science and Technology, 1999 30(2), 100-108. <https://doi.org/10.1080/027868299304705>
- Ch.Battssetseg. *Practicum of Microbiology*, Улаанбаатар хот, 2007. <https://doi.org/10.1080/027868299304705>
- El-Shahawy, A. A. G., Abo El-Ela, F. I., Mohamed, N. A., Eldine, Z. E., & El Roubay, W. M. A. *Synthesis and evaluation of layered double hydroxide/doxycycline and cobalt ferrite/chitosan nanohybrid efficacy on gram positive and gram negative bacteria*. Materials Science and Engineering. 2018, C, 91, 361-371. <https://doi.org/10.1016/j.msec.2018.05.042>
- B. H. *Delivery system for berberine chloride based on the nanocarrier ZnAl-layered double hydroxide: Physicochemical characterization, release behavior and evaluation of anti-bacterial potential*. International Journal of Pharmaceutics. (2016), 515(1-2), 422-430. <https://doi.org/10.1016/j.ijpharm.2016.09.089>
- Jin, S.; Fallgren, P. H.; Morris, J. M.; Chen, Q. *Removal of Bacteria and Viruses from Waters Using Layered Double Hydroxide Nanocomposites*. Sci. Technol. Adv. Mater. 2007, 8 (1-2), 67-70. <https://doi.org/10.1016/j.stam.2006.09.003>
- Theiss, Frederick L. Ayoko; Godwin A. Frost; Ray L. *Synthesis of layered double hydroxides containing Mg<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup> and Al<sup>3+</sup> layer cations by co-precipitation methods - A review*. Applied Surface Science. 2016, 383, 200-213. <https://doi.org/10.1016/j.apsusc.2016.04.150>