ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article DOI: 10.5281/zenodo.18105285

An investigation on the synthesis, characterization and antibacterial activity of silver nanoparticles (Ag-NPs)-modified montmorillonite

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Received	16.09.2025
Revision Requested	21.11.2025
Last Revision	01.12.2025
Accepted	08.12.2025
Publication Date	31.12.2025

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Cite this article: Öztürk SZ, Semerci AB, Topçu S, Tuna M, Çalışkan F. An Investigation on the Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles (Ag-NPs)-Modified Montmorillonite. *NanoEra*. 2025;5(2):42-49

ABSTRACT

In this study, silver nanoparticle (Ag-NP) modified sodium montmorillonite (Na-MMT) was synthesized via a sol–gel-assisted ion-exchange approach. During the process, Ag^+ cations were intercalated into the interlayer spaces of Na-MMT, leading to the successful preparation of Ag-enriched organoclays. Structural modifications were verified by Fourier-transform infrared spectroscopy (FTIR), while scanning electron microscopy (SEM) revealed well-dispersed silver nanoparticles on the clay surface. Energy-dispersive X-ray spectroscopy (EDS) provided additional confirmation of silver incorporation within the layered framework. The antibacterial performance of six Ag-MMT formulations was assessed via the Kirby–Bauer disk diffusion assay against *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium, Staphylococcus epidermidis,* and *Staphylococcus aureus*. The most effective sample exhibited a maximum inhibition zone of 13.5 mm against *S. typhimurium*, while a 2% Ag loading was found to be the minimum effective concentration for broad-spectrum antibacterial activity. This study is the first to produce silver composite (Ag-MMT) nanomaterials using local Karakaya (Türkiye) bentonite. The findings indicate that Ag-MMT nanocomposites possess potent antibacterial properties. The study determined that the highest zone of inhibition occurred against *S. typhimurium*, a bacterium that causes serious infections in humans and poses a public health risk as a food chain carrier. Ag-MMT nanocomposites could be used in water treatment, food packaging, and biomedical applications. Furthermore, an innovative protection strategy for smart agriculture could be developed to combat the risks posed by *S. typhimurium* in soil pathogen control. These findings highlight Ag-MMT as a promising nanocomposite for antimicrobial applications.

Keywords: Silver, Organoclay, Modification, Cation Exchange, antibacterial

INTRODUCTION

Clay minerals are layered aluminosilicates that combine a unique set of structural, chemical, and physicochemical features, such as high surface area, tunable ion-exchange capacity, non-toxicity, and excellent adsorption properties, making them attractive for diverse applications ranging from catalysis to biomedical use.[1–3]

Natural and low-cost, montmorillonite is a good matrix medium for metal ions. It has a 2:1 dioctahedral and is composed of a symmetrical structure with wide gaps between two tetrahedral silica layers, consisting of an octahedral alumina layer. It has a large specific surface area and small particle structure, great efficiency for adsorption and cation exchange capacity.[4–7] Montmorillonite is a fine-grained, layered aluminosilicate mineral characterized by its high hydrophilicity. This property arises from the structural charge imbalance created by non-stoichiometric substitutions within the crystal lattice, which generates an excess negative charge.

These charges are compensated by exchangeable cations located in the interlayer galleries, giving the material remarkable ion-exchange capacity. The general chemical composition of montmorillonite can be represented as $(Ca, Na)(Mg, Al, Fe)_2(Si, Al)_4O_2 \cdot nH_2O$.[1]

The immobilization and synthesis of metal nanoparticles on solid supports, particularly montmorillonite clays, offer an effective route for tailoring particle size and improving their practical applicability. Montmorillonite's exceptional swelling ability and strong adsorption capacity make it an attractive host material for incorporating antibacterial-active nanoscale metals within its interlayer spaces. Such modifications not only stabilize the nanoparticles but also enhance their distribution, thereby broadening the functional potential of clay-based nanocomposites in various technological and biomedical applications.[8,9] In the literature studies, montmorillonite (MMT) clay, which has a maximum 2:1 layer structure, was used. This is because there is water between the layers of MMT clay. In the presence of water, interlayer cations attract water molecules and separate the clay layers from each other in a process generally described as clay hydration or clay swelling.[4,5,7,10,11]

When there are Ca^{+2} ions between the layers, the layers are close to each other, when there are Na^{+} ions, the gap between the layers widens and it becomes possible for a polymer to bind to the free hydroxyl groups there. These ions can be replaced by organic cations in the environment if the clay can be modified organically. As a result of this displacement, the polarity of the layers changes and as a result, the interlayer expands. These clays, which have an expanded structure, create a potential for the synthesis of nanocomposite materials.[12–14] Alumina octahedron and silica tetrahedron structure, molecular structure of montmorillonite containing Na ion as exchangeable cation, silver-doped montmorillonite illustration with interlayer gallery, and different angle view of montmorillonite clay are given in Figure 1.

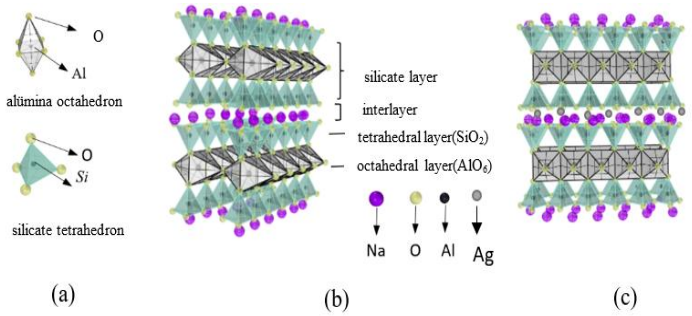


Figure 1. a) Alumina octahedron and Silica tetrahedron structure. **b)** Molecular structure of montmorillonite containing Na ion as exchangeable cation. **c)** Silver-doped montmorillonite illustration with interlayer space and different angle view of montmorillonite clay

The growing prevalence of antibiotic-resistant bacteria has become a critical global health concern, prompting extensive research over the past decade to design novel antibacterial materials capable of addressing this challenge. For this purpose, the scientific world is trying to add bactericidal character by incorporating metals with known antibacterial properties, such as silver, into the structure of composites.[15,16]

Silver is a highly preferred antibacterial agent due to its chemical stability, longevity and high antibacterial properties. In some cases, silver particles used alone can cause various adverse effects related to the leaching and aggregation phenomenon, which can create environmental problems and limit their antibacterial activity.[17–19] To eliminate these disadvantages and exhibit antibacterial activity for a longer time, silver particles could be embedded into a solid matrix. Among these approaches, the incorporation of silver into host matrices has resulted in a promising class of materials that retain the potent antibacterial activity of silver while offering enhanced applicability across diverse fields. In addition, such systems are often considered more environmentally sustainable compared to conventional antimicrobial agents. Recently produced metal matrix materials are an alternative to traditional antibacterial materials and are used in many industrial areas such as medical instruments, food storage containers, and agricultural applications.[20–23]

As a result of the important properties of montmorillonite, researchers have increased their efforts to combine its properties with those of other inorganic/organic materials to produce new hybrid materials.[24,25]

Consequently, developing Ag-MMT hybrid materials offers a sustainable pathway for producing antimicrobial agents applicable in biomedicine, food preservation, and environmental remediation. This work aimed to incorporate Ag nanoparticles into Na-MMT via a sol–gel-assisted ion exchange process, to characterize the resulting composites using FTIR, SEM, and EDS techniques, and to evaluate their antibacterial performance against six clinically relevant bacterial strains.

MATERIALS and MERHOD

The metallic silver powder was supplied by Nanokar Nanotechnology Co., while sodium montmorillonite was received by Karakaya Bentonit Co. Distilled water and ethanol were employed for all cleaning processes. All solutions were prepared by the wet method. The morphology of the powder particles was characterized using a scanning electron microscopy (JEOL, JSM-6060LV). The spectroscopic characterization of the synthesized substances was carried out by FT-IR analysis (Perkin Elmer UATR Two diamond ATR). To reveal the elemental distribution of the synthesized powders, an Energy dispersive spectroscopy (EDS) was used. Mixing processes with heating were conducted in a Hydra-brand ultrasonic bath.

Preparation of Organoclay Samples

As a method, modified precursor montmorillonite with different silver powder ratios was produced using the sol-gel method. Silver-doped montmorillonite (Ag-MMT) was prepared by an ion exchange reaction. 4.3 g of Na-MMT powder was stirred in 50 mL of deionized water at 50°C for 2 hours. Thus, the layered structure of Na-MMT was separated, and the ratios of 0.156 meq, 0.469 meq, 0.626 meq, 1.28 meq, 1.616 meq and 1.96 meq (G1, G2, G3, G4, G5, G6, respectively) were added to the aqueous solution for the silver ions to substitute. The silver powder was put in the solutions and mixed at 60 °C for 2 hours. The formed gel was kept in a drying oven at 120 degrees for 24 hours to remove the water in the structure and bring the layers of silver-doped MMT closer together. The resulting cake-like structure was ground into powders in a vibratory disk mill, and subsequently examined by SEM, FTIR, and EDS, followed by antibacterial testing.

Preparation of Organoclay Samples

Antimicrobial activity was assessed using the disk diffusion method against *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *E. coli* ATCC 8739, *S. typhimurium* ATCC 14028, *S. epidermidis* ATCC 12228, and *S. aureus* ATCC 29213. Suspensions at 0.5 McFarland density were prepared using a densitometer from fresh strains that were activated overnight. Different percentages of Ag-modified MMT were dissolved with DMSO, and 20 µL were absorbed into sterile discs (6 mm in diameter).

For the antibacterial evaluation, standardized suspensions of microorganisms, adjusted to a defined cell density, were inoculated onto Mueller–Hinton agar plates using sterile swabs. Discs impregnated with the test materials were then aseptically positioned on the agar surface. Following 24 hours of incubation at 37 °C, the inhibition zones were measured with a digital caliper to quantify antibacterial activity. In this assay, discs impregnated with dimethyl sulfoxide (DMSO) served as the negative control, whereas gentamicin-loaded discs were employed as the positive control. All experiments were performed in triplicate and results are given as mean±standard error.

RESULTS

In this study, the silver-added modified montmorillonite powder was synthesized and investigated. In Table 1[26], the chemical content of montmorillonite used in the experimental study is given as per cent by weight (wt.). Na-MMT and six different Ag-MMT bond structures were examined in the FT-IR analyzed as an elemental distribution.

Table 1. Chemical content of montmorillonite.[26]

	wt %
SiO_2	60-62
MgO	1.8-2
Na_2O	2.5-3
Al_2O_3	17-18
Fe_2O_3	3-3.5
K_2O	0.9-9.5
CaO	3.5-4

FT-IR Analysis Findings

The FT-IR spectra of Na Montmorillonite (Na-MMT) and Silver-doped montmorillonite (Ag-MMT) in the range of 4000 to 400 cm-1 are displayed in Figure 2. The spectra clearly showed the existence of vibrational bands characteristic of montmorillonite.[23]

Vibration bands of MMT; OH stretch band in 3631–3444 cm^{-1} region, Si–O–Si tensile band region in 1091–1039 cm^{-1} range and Si–O stretch vibration band at 795 cm^{-1} were observed.[24] A well-defined peak associated with the stretching mode of the –OH group coordinated to Al cations appeared at 3631 cm^{-1} in the high-frequency range.[27] When the FTIR analyses were compared, the functional group table did not change as expected with the addition of silver nanoparticles. Therefore, specific peaks of montmorillonite were observed in all FT-IR spectra.[28] This finding suggests that Ag-NP loading does not disrupt the MMT silicate lattice, with Ag species adsorbing primarily to the clay surface and/or interlayers. No new functional group formation was detected, except for limited intensity changes observed in FTIR; the presence and distribution of Ag were confirmed by XRD and SEM–EDX analyses.[29]

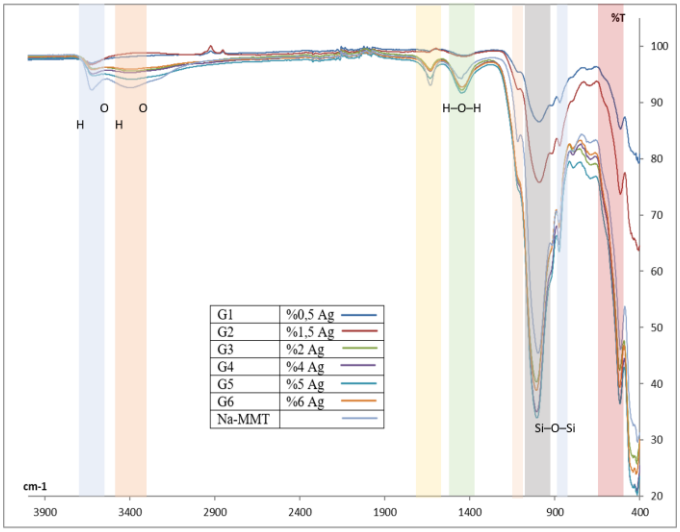


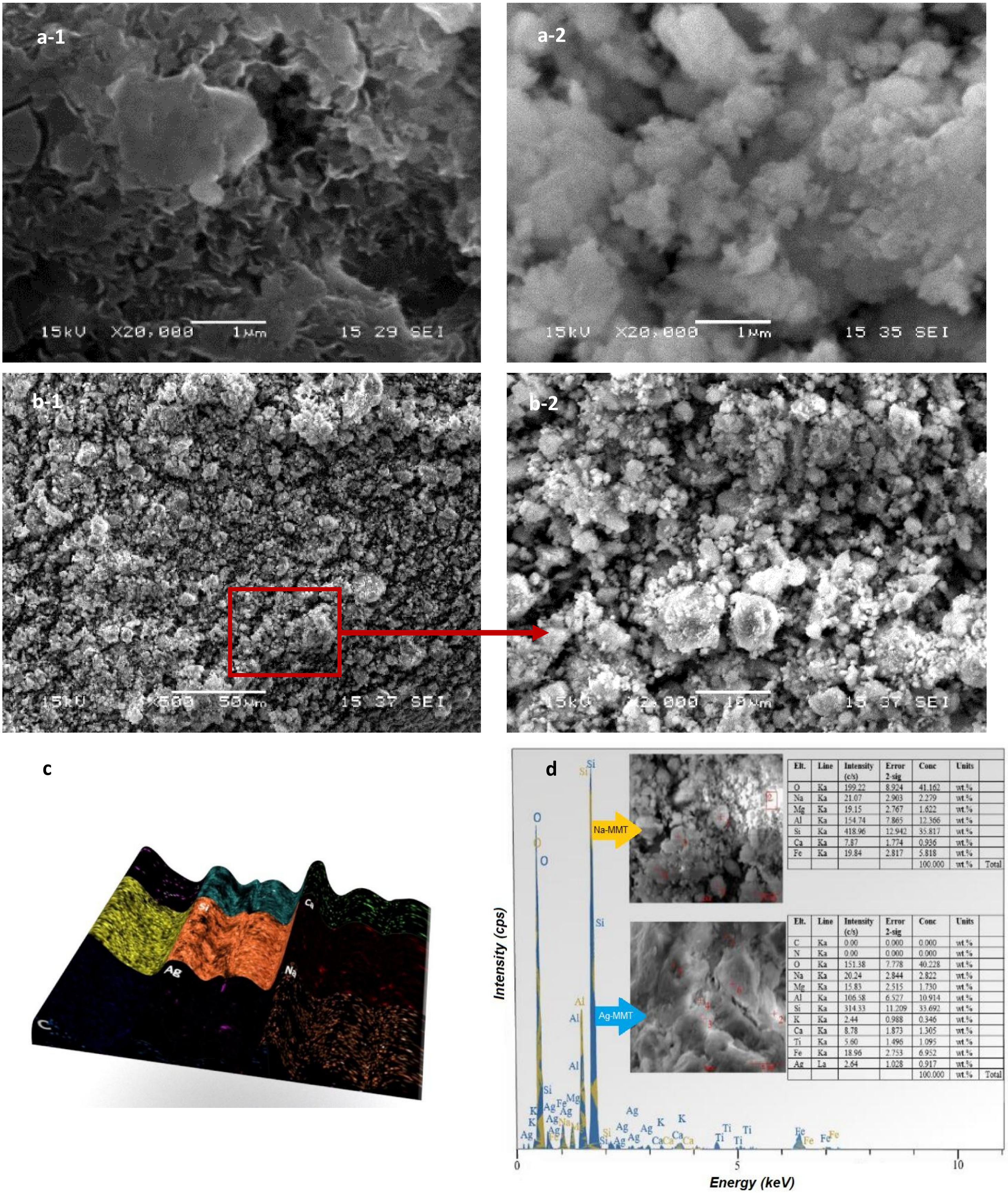
Figure 2. Na-MMT and Ag-NP FTIR spectra

Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) Analysis

SEM-EDX analyses were performed to determine the morphological structures and elemental compositions of Ag-NPs (Figure 3). Ag-NPs were found to be spherical in SEM micrographs. As given in Figure 3 (a) and (b), few particles were seen to exhibit face-to-face, end-to-end, and corner-to-corner clusters. The morphologies of these particles showed that the sample had a layered structure; more particles appear to be irregularly agglomerated in Figure 3 (a-1 and a-2) in the form of voluminous flakes.[30] The difference in appearance between Figure 3 a-1 and a-2 was observed on the surfaces of clay minerals, which can be referenced to the formation of silver nanoparticles at the edges in Ag-MMT compared to Na-MMT.[31] In EDX analysis (Figures 3 c and d), Na montmorillonite and Ag-MMT data were given together, Ag-MMT indicated with green peaks and green arrows, values and image indicated by red peaks and red arrows were indicated as MMT. Silver peaks in EDX data indicate the presence of Ag-NPs. Weak peaks from titanium and iron are due to pollution from the raw material.[32] Figure 3 (d). EDS element mapping confirmed the presence of Ag and an elementally homogeneous structure in the nanocomposite Ag-MMT.

Antibacterial Activity Analysis

Antibacterial activities of prepared Ag-modified MMTs on test bacteria (Gram-negative *E.coli*, and Gram positive *E. faecalis, B. subtilis, S. aureus, S. epidermidis,* and *S. typhimurium*) were investigated using the disc diffusion method. Study results are presented in Table 2 and Figure 4.



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units	
O	Ka	199.22	8.924	41.162	wt.%	
Na	Ka	21.07	2.903	2.279	wt.%	
Mg	Ka	19.15	2.767	1.622	wt.%	
Al	Ka	154.74	7.865	12.366	wt.%	
Si	Ka	418.96	12.942	35.817	wt.%	
Ca	Ka	7.87	1.774	0.936	wt.%	
Fe	Ka	19.84	2.817	5.818	wt.%	
				100.000	wt.%	Total

Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units	
C	Ka	0.00	0.000	0.000	wt.%	
N	Ka	0.00	0.000	0.000	wt.%	
O	Ka	151.38	7.778	40.228	wt.%	
Na	Ka	20.24	2.844	2.822	wt.%	
Mg	Ka	15.83	2.515	1.730	wt.%	
Al	Ka	106.58	6.527	10.914	wt.%	
Si	Ka	314.33	11.209	33.692	wt.%	
K	Ka	2.44	0.988	0.346	wt.%	
Ca	Ka	8.78	1.873	1.305	wt.%	
Ti	Ka	5.60	1.496	1.095	wt.%	
Fe	Ka	18.96	2.753	6.952	wt.%	
Ag	La	2.64	1.028	0.917	wt.%	
				100.000	wt.%	Total

Figure 3. SEM-EDX graphs of Na-MMT and Ag-MMT; **(a-1)** Na-MMT morphological appearance, **(a-2)** Ag-MMT morphological appearance, **(b-1)** Ag-MMT 500X and **(b-2)** 2.000X SEM surface image, **(c)** Ag-MMT Element Distribution, **(d)** Na-MMT and Ag-MMT EDS analysis peaks

Table 2. Antibacterial activity of Ag-modified MMTs prepared in different percentages.

	Zone of Inhibition on Bacterial Test (mm) (±SD)					
	S. aureus	*S. epidermidis*	*E. faecalis*	*E. coli*	*B. subtilis*	*S. typhimurium*
0.5% (G1)	0	0	0	8±0.1	8±0.1	8.1±0.2
1.5% (G2)	0	8.3±0.7	0	8.5±	8±0.1	8±0.1
2% (G3)	11.5±0.3	9±0.1	8.7±0.9	8±0.1	8±0.1	13.5±0.7
4% (G4)	9.7 ±0.6	8±0.1	6.5±0.3	8.7±0.1	8.5±0.1	12±0.1
%5 (G5)	10±0.1	8±0.1	7±0.1	8±0.1	8.5±0.5	12±0.1
%6 (G6)	9.5±	8±0.1	8±0.1	8±0.1	8±0.1	11±0.1
Na-MMT	0	0	0	0	0	0
N. control	0	0	0	0	0	0
Gentamicin	21	20.3	18.5	17	20	20.5

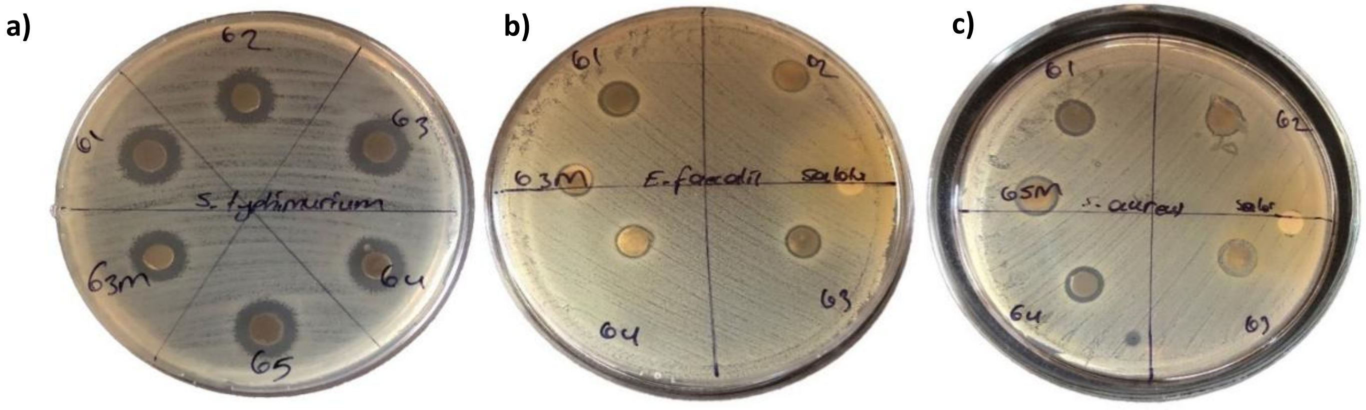


Figure 4. Antibacterial activities of Ag-MMTs at various concentrations on **a)** *S. typhimurium*, **b)** *E. faecalis*, **c)** *S. aureus*.

The presence of an inhibition zone in the Petri dishes clearly demonstrated the antibacterial effect of Ag-modified MMTs on the test bacteria. It was determined that raw Na-MMT, not treated with Ag, had no antibacterial effect, whereas the modified Ag MMT complexes formed had activity on all test bacteria used. The antibacterial activity of Ag-modified MMT is derived from the crystal structure of the smectite group, which consists of two octahedral alumina layers located between two tetrahedral silica layers of MMT. The composite produced by the interaction of this structure with cationic Ag and trapping these ions in the MMT content exhibited antibacterial activity. FT-IR results also support this connection. While it was observed that increasing silver concentration did not cause an increase in antibacterial activity, the highest antibacterial activity was detected in MMT produced with 2% silver. When the antibacterial activity of 2% Ag modified MMT, especially on *S. typhimurium* (13.5 mm) and *S. aureus* (11.5 mm), was compared with standard gentamicin, it was determined that it showed effective antibacterial properties.

In a study evaluating the antibacterial activity of Ag-containing coatings prepared with hydroxyapatite, it was reported that the coating containing 3% by weight Ag had excellent antibacterial properties and showed minimal cytotoxicity.[27] Therefore, it can be thought that 2% Ag-MTT with antibacterial activity obtained in our study is not toxic to cells.

In a study conducted with montmorillonite clay powder, a ceramic composite containing 14.5% silver in its structure was produced by an ion exchange process (mixing with silver nitrate solution). It was reported that this composite showed 99% antibacterial activity on *S. aureus*.[33] In this study, it was evaluated that the Ag MMT composite synthesized with a lower silver concentration (2%) showed antibacterial activity. Depending on the structural features of MMT employed in our investigation, silver concentration, and antibacterial activity technique, different findings were obtained. Similar to present work, the antibacterial activity technique reported that nanocomposites containing Ag-Clay, which they synthesized without employing reducing agents, exhibited antibacterial activity in *E.coli* (8 mm on top) and *S. aureus* (12 mm).[34]

Salmonella typhimurium, the most potent antibacterial agent in this study, poses a direct threat to humans as a serious infectious pathogen. It is also the bacterium most frequently associated with foodborne illness. Literature indicates that *S. typhimurium* can enter raw agricultural products such as tomatoes via leaves or stems, colonize the interior of the plant, and multiply at high densities within the plant without causing symptoms. This suggests that the pathogen acts as a carrier in the food chain, posing a serious risk to public health. Therefore, the high efficacy of Ag-MMT against S. typhimurium highlights its potential to control the spread of the pathogen in agricultural products.[6]

Numerous studies in the literature report that AgNP-doped materials exhibit antibacterial and antifungal activity against bacteria, suggesting that these properties are due to the AgNPs' ability to directly bind to microorganisms, thus providing a direct biocidal effect.[35,36] Furthermore, some studies indicate that Ag^+ ions have the ability to disrupt DNA and proteins by blocking their structures. Furthermore, some studies have shown that Ag^+ cations, which can be released by AgNPs, have a strong affinity for organic compounds containing sulfur and phosphorus atoms. There is also data indicating that they disrupt membrane structure and trigger the formation of ROS.[37,38]

CONCLUSION

This study demonstrates the potential applicability of hybrid materials derived from natural resources, which are gaining increasing importance across diverse fields. Silver-doped montmorillonite (Ag-MMT) composites were successfully synthesized from domestic raw materials via the sol–gel method. Structural analyses confirmed that the intended nanocomposites were obtained, and all prepared samples exhibited antibacterial activity against six bacterial strains. Notably, the composite containing 2% Ag showed the most consistent performance, effectively inhibiting bacterial growth and generating clear inhibition zones. An important advantage of Ag-based composites over conventional antibiotics is the absence of reported bacterial resistance.[39] Overall, Ag-MMT nanocomposites with strong antibacterial properties were produced through a simple and efficient approach, highlighting their potential for practical applications such as water purification and food packaging.

Peer-review: Externally peer-reviewed.

Author Contributions: Data Collection and Processing - S.Z.Ö.; Analysis - A.B.S.; Literature Search - A.S.T.; Writing Manuscript S.Z.Ö.; Supervision - M.T. and F.Ç.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Use of Artificial Intelligence: The author(s) declare that Artificial Intelligence is not used except for grammar check.

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