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Green Synthesis of Silver Nanoparticles Using Hibiscus Extract for the Remediation of Polluted Diyala River

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ABSTRACT

In this study, biological and chemical pollutants from various points of Diyala River (wastewater, sewage, orchard water, running water, and liquefied water) were treated by synthesizing silver nanoparticles (Ag NPs) by green synthesis method using hibiscus flower extract. The synthesized silver nanoparticles were characterized using X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), and Fourier transform infrared spectroscopy (FTIR). XRD technique showed that the silver nanoparticles were polycrystalline with the predominant direction (122) (111), (200) (220) (311), while (EFSEM) showed that the silver nanoparticles were spherical. The biological properties of total aerobic bacteria (TPC) and chemical properties, including p, total dissolved salts (TDS), and total suspended solids (TSS) of the water, were tested before and after treatment with silver nanoparticles. All rates of biological and chemical properties decreased after treatment. Silver nanoparticles also showed excellent antibacterial activity against Gram-negative pathogenic bacteria (*Pseudomonas, Acinetobacter, Cholera, and Escherichia coli*) commonly found in contaminated water by adding different ratios of silver nanoparticles and the best concentration to obtain a clear inhibition zone relative to other concentrations was 100%.

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1. INTRODUCTION

The study of water pollution is considered very important to most researchers and interested parties, and its importance lies in the fundamental changes it causes in the lives of humans, animals, plants, soil, and the environment in general. The research dealt with the study of biological and chemical pollutants that occur due to the pollution of the Diyala River with sewage, as well as industrial and agricultural waste [1]. Biological pollution results from microorganisms such as bacteria and viruses found in rivers from sewage and industrial and agricultural waste, as they affect the digestive system. Symptoms of waterborne diseases include severe diarrhea, nausea, vomiting, and possibly jaundice, as well as headache and fatigue [2]. Bacteria in polluted water include *Salmonella, Shigella, Escherichia coli, cholera, Pseudomonas aeruginosa,* and *Acinetobacter*. A group of pathological cases have been diagnosed with bacteria, including (*Pseudomonas aeruginosa, Acinetobacter, Escherichia coli,* and *cholera*). *Pseudomonas* aeruginosa is a Gram-negative, motile, non-feeding, rod-shaped bacterium, 1 to 5 μm long and 0.5 to 1.0 μm wide. A facultative aerobe grows by aerobic and anaerobic respiration at 37 °C [3]. *Escherichia coli* (*E. coli*) is a Gram-negative bacterium and a type of fecal coliform bacteria that is commonly found in the intestines of animals and humans. It is so small that it cannot be seen without a microscope. These microorganisms are excreted in fecal material or feces; their spread is called the "fecal-oral" transmission route. Contaminated food and water are the most common routes of exposure to *E. coli* [4]. Also, bacteria of the genus *Acinetobacter* are Gram-negative, non-motile, non-fermentative, and non-robust bacteria. The cells may be spherical, especially in the stationary growth phase. Most strains of these organisms grow at temperatures ranging from 20 °C to 37 °C. Colonies are generally unpigmented, pale yellow or grayish white. The bacteria are strictly aerobic, catalase-positive, and oxidase-negative [4]-[5]. Finally, *Vibrio cholerae* is a Gram-negative bacterium that infects humans through unsafe water and food contaminated with human feces containing the bacteria. [6]. Chemical pollution results from excessive amounts of dissolved salts, acids, fluoride, metals, organic matter, fertilizers, and pesticides[1].

This study aims to prepare silver nanoparticles using the green synthesis method and study their effect on the biological and chemical properties of polluted water from different points of the Diyala River, which agrees with [7]. Through biological application, it was observed that silver nanoparticles have antibacterial activity against a group of Gram-negative bacteria such as (*Pseudomonas*, *Acinetobacter*, *Cholera*, and *Escherichia coli*) by incorporating silver nanoparticles into the cell membrane, which causes the leakage of materials into the cells and eventually leads to cell death [8]-[9].

2. EXPERIMENTAL PART

2.1 Sample Collection

Five samples were collected from the Diyala River from different points, including puncture water, sewage water, orchard water, running water, and liquefied water, as shown in Figure 1, using a 100 ml glass beaker prepared for this purpose. The biological properties represented by total aerobic bacteria (T.P.C) and the chemical properties defined by the potential of hydrogen meter (PH) (AD1200 PH/mV/ISE) and temperature meter mV, total dissolved salts (TDS), and total suspended solids (TSS) of the water samples were examined in the laboratories of the Environment Directorate in Diyala Governorate.

Figure 1. Samples from Diyala River
(A-puncture water, B-sewage water, C-orchard water, D-running water, and E- liquefied water)

2.2 Preparation of Silver Nanoparticles by Hibiscus Flower Extract

Silver nanoparticles were prepared using hibiscus flower, where hibiscus flowers were first ground and sieved using a 38 µm fine sieve, then 2 grams of them were added to 100 ml of deionized water. The solution was placed on a magnetic stirrer for 1 hour at 50 °C until it turned into a dark red solution, and after 1 hour of preparing the solution, it was filtered. Then, a silver nitrate solution was prepared by mixing 1.7 gm of silver nitrate at a concentration of 0.1 mM with 100 ml of deionized water. The solution was placed on a magnetic stirrer for 2 hours at 70 °C. Finally, 3 ml of hibiscus solution was added dropwise to the silver nitrate solution, and 5 µl of hydrochloric acid was added to enhance solubility. Finally, a dark gray solution was obtained, which is the bio-manufactured silver nanoparticles, as shown in Figure 2. Silver nanoparticles were characterized using various analytical techniques, including X-ray diffraction (XRD) with PAN analytical Aeris X-ray diffractometer (Cu Kα1 radiation and wavelength of 1.54059 Å), field emission scanning electron microscopes (FE-SEM) inspect TM F50, and Fourier transforms infrared spectroscopy (FTIR) Model Spectrum Two Perkin Elmer.

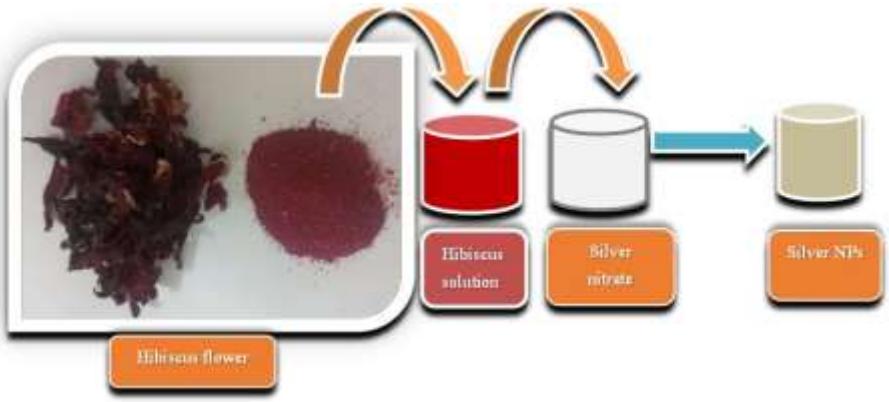

Figure 2. Preparation stages Ag NPs

2.3 Treatment by Ag NPs

1 ml of sliver nano solution was added to 100 ml of the water sample previously collected from Diyala River points and then placed on an ultrasonic device for one hour at room temperature. The samples were left to stagnate for 24 hours after the ultrasonic was completed. Then, the water was filtered after stagnation using filter paper, as shown in Figure 3. The biological and chemical properties of the samples were tested after treatment by Ag NPs.

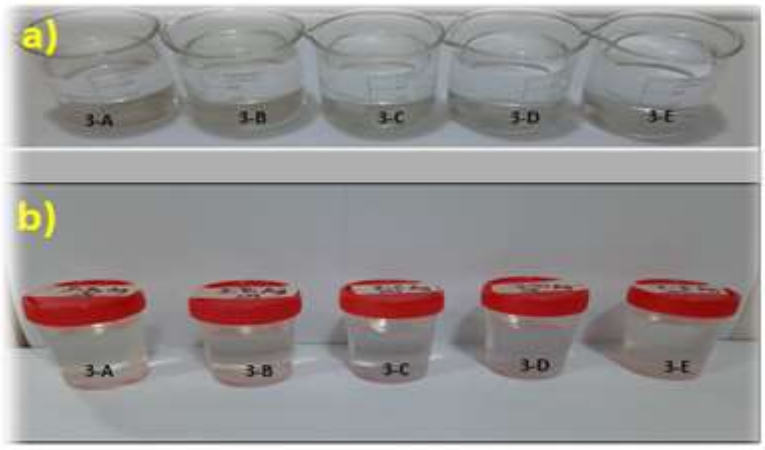

Figure 3. Diyala River samples: a) before treatment and b) After treatment by Ag NPs

2.4 Anti-Bacterial Assay

Several types of Gram-negative bacteria were isolated and diagnosed from the waters of the Diyala River. This well-diffusion investigation of antibacterial activity was conducted using the bacteria (CFU/ml) from 8 new colonies that were suspended in 5 ml of normal saline (1.5 × 10 bacteria). A portion of the bacterial suspension was transferred to a sterile cotton swab and then carefully and uniformly dispersed on Mueller-Hinton agar medium. The antibacterial ability of Ag NPs against Gram-negative bacterial strains usually found in polluted water (*Pseudomonas*, *Acinetobacter*, *Escherichia coli*, and *Cholera*) has been studied using agar diffusion assay [10]-[11]. About 20 ml of Mueller-Hinton (MH) agar was aseptically poured into sterile Petri dishes. Bacterial species were collected from their cultures using a sterile wire loop [12]. After culturing the organisms, 6-mm diameter wells were drilled onto the agar plates using a sterile tip. In drilled wells, different concentrations (12.5, 25, 50, and 100%) of samples of Ag NPs were added. The culture plates containing the Ag NPs and test organism samples were incubated for 24 hours at 37°C before measuring and recording the average areas of inhibition diameter [13]-[14].

3. RESULTS AND DISCUSSION

Silver nanoparticles were synthesized using the green synthesis technique for contaminated water treatment, and XRD and FESEM measured their structural and morphological properties. Measuring the X-rays, as shown in Table 1, indicated that the silver nanoparticles are polycrystalline, as well as the appearance of silver nanoparticles in the dominant direction (122) (111), (200) (220) (311), the crystallite size was calculated from a well-known Scherrer equation ($D_{hkl} = K\lambda/(B_{hkl}cos\theta)$) with an FCC structure of the synthesized silver nanoparticles, where the crystallite size (69 nm) of the dominant direction (200) was as shown in Figure 4. The organic compound present in the extract of hibiscus flowers forms an unstable crystalline band [15].

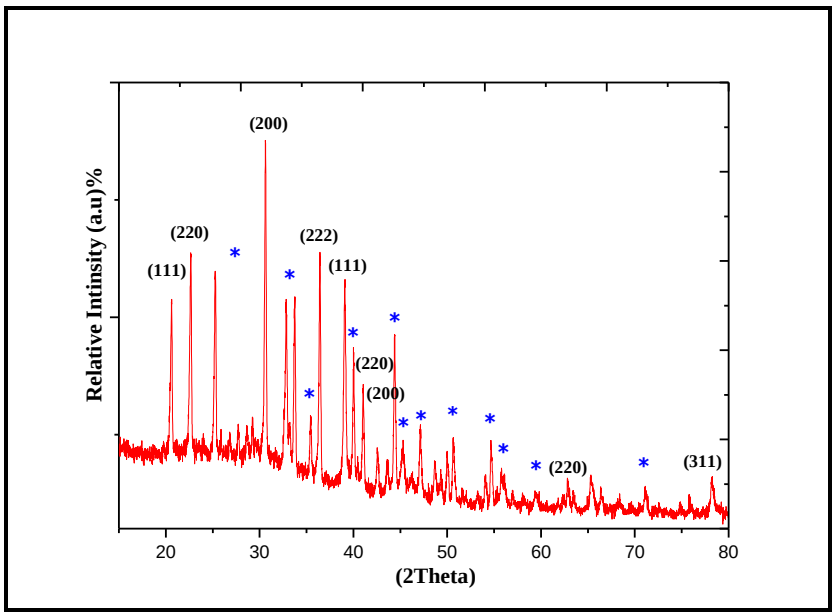

Figure 4. XRD spectra of Ag NPs

Table 1. XRD results of Ag NPs

No.	Pos. (2θ°) Ag	Pos. (2θ°) JCPDS file number 00-003-0921	hkl
1	20.6315	21.1	111
2	22.7025	21.95	220
3	30.6323	30.1	200
4	36.4509	36.90	222
5	39.0793	38.2	111
6	42.5624	43.9	220
7	43.6304	43.4	200
8	62.8218	61.2	220
9	78.19	78	311

The results of a field emission scanning electron microscope (FESEM) were analyzed to characterize the shape and size of the Ag NPs. The fabricated nanoparticles ranged in size from 23.19 nm to 133.1 nm, as in Figure 5; it was found that the average particle size of the silver particles is 52.532 nm with a spherical shape [16].

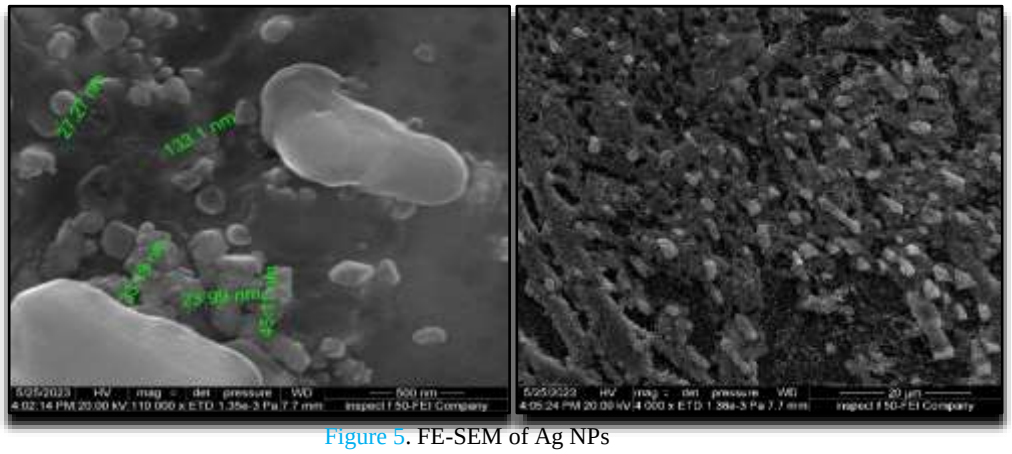

Figure 5. FE-SEM of Ag NPs

The functional groups in silver nanoparticles have been mapped using FTIR spectroscopy, and possible biomolecules responsible for reducing Ag+ ions and covering Ag NPs have been identified and mixed using hibiscus flower extract. FTIR spectrum of the silver nanoparticle was recorded in the range of 483.91-3852.07 cm^{-1}, as shown in Figure 6. Several significant absorption peaks were observed; the IR spectra of Ag NPs showed broad absorption bands at a range of 3852.07-3745.08 cm^{-1}, which corresponds to O-H stretching bands that are attributed to compound phenolic, and at 3446.32 cm^{-1}, which corresponds to O-H to groups function hydroxyl [17], and 2923.86 cm^{-1} which corresponds O-H that attributed to compound carboxylic acids [18], and at 2853.26,2064.79 cm-1 which corresponds C-H that attributed to compound alkane [19], and 2397.56 cm^{-1} which corresponds to C=C to groups alkyne [20], and at 1635.89 cm^{-1} which corresponds to C=O that attributed to compound carboxyl [20], and at 1383.98 cm^{-1} which corresponds to C-H to group hydrocarbons [21], and at 1083 cm^{-1} which corresponds C-O that attributed to compound carboxyl [22],

1031.39 cm^{-1} which corresponds C-O that attributed to compound carboxylic acids [16], and at 745.86 cm^{-1}, 483.91 cm^{-1} which corresponds Ag-O that attributed to compound metal oxide [21], [16]. Table 2 shows FTIR results indicating the wave number as a function of the transmittance.

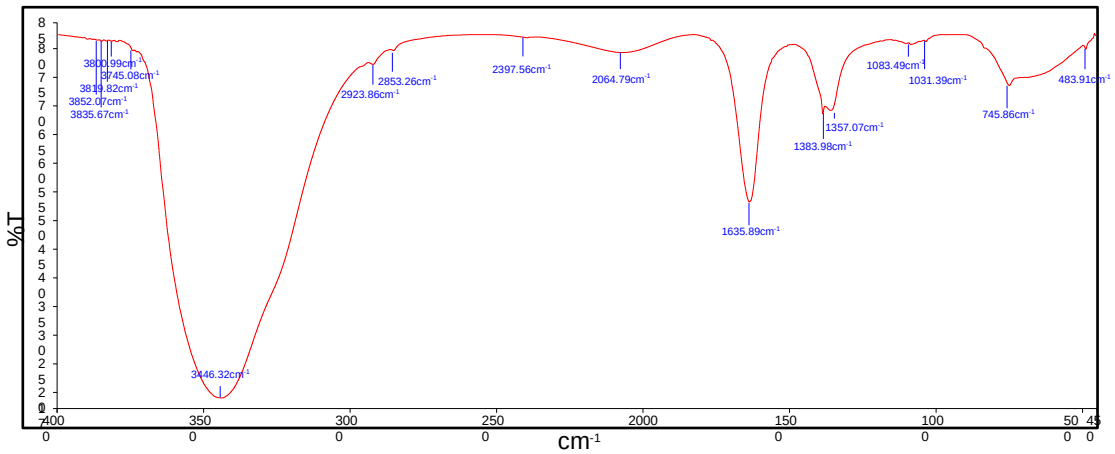

Figure 6. FTIR of Ag NPs

Table 2. FTIR Results of Ag NPs

Peak Number	Wavenumber (cm^{-1})	Transmittance (a. u.)	Group
1	3852.07	81.55	O-H
2	3835.67	81.56	O-H
3	3819.82	81.54	O-H
4	3800.99	81.43	O-H
5	3745.08	79.87	O-H
6	3446.32	18.82	O-H
7	2923.86	77.46	O-H
8	2853.26	79.90	C-H
9	2397.56	82.15	C=C
10	2064.79	79.53	C-H
11	1635.89	53.27	C=O
12	1383.98	68.78	C-H
13	1357.07	69.39	C-O
14	1083.49	80.94	C-O
15	1031.39	81.47	C-O
16	745.86	73.74	Ag-O
17	483.91	80.15	Ag-O

The water samples collected from different points of the Diyala River were tested for the chemical properties shown in Table 3, including the pH scale, which is used to express the acidic or basic strength of the water. The PH scale ranges from 0 to 14. PH 7 is neutral, indicating a balance between acidic and basic elements; values below 7, which reach 0, indicate increased acidic strength. Values above 7 indicate increased basic strength as the pH approaches 14 [23]. We note that the PH of the collected samples became basic after treatment; as for the total dissolved salts (TDS), which is a measure of the combined content of all inorganic and organic materials or salts present in the water [24], we note that it decreased after treatment because the silver nanoparticles have a high surface area and can absorb or bind to other molecules present in the water [25], such as organic materials, colloids or other pollutants. By absorbing these particles, silver nanoparticles can facilitate their removal from water. This absorption process can reduce the concentrations of dissolved solids [25]. Total suspended solids (TSS), which include a mixture of clay, silt, and some microorganisms such as plankton, and organic and inorganic matter [26], decrease after treatment for several reasons, including that the size of Ag NPs is relatively more significant than the dissolved pollutants, as they may aggregate and form larger particles; these aggregates can settle due to gravity, which leads to the removal of suspended particles from the water column. As a result, the concentration of TSS decreases.

Table 3. Chemical properties of Diyala River samples

Form site		PH		TDS		TSS	
Points Symbol	Points Site	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
A	Puncture water	6.2	8	1436	949	65	15
B	Sewage water	7.1	8	975	732	35	8
C	Orchard water	7	7.8	383	327	20	9
D	running water	7.5	8	417	351	36	18
E	Liquefied water	6.9	7.8	396	308	10	3

As for the biological characteristics represented by the total aerobic bacteria (TPC) as shown in Table 4, which means bacteria that grow in the presence of oxygen [23], we note that aerobic bacteria decreased after treatment; this is because silver has antibacterial properties, as it inhibits the growth of bacteria by affecting their cell membranes and interfering with the vital processes inside the cells [27], thus reducing the number of aerobic bacteria in the treated water.

Table 4. Biological properties of Diyala River samples

Form site		T.P.C	
Points Symbol	Points Site	Before Treatment	After Treatment
A	Puncture water	800	25
B	Sewage water	280	18
C	Orchard water	706	26
D	running water	500	17
E	liquefied water	25	3

Through the biological application, it was observed that silver nanoparticles synthesized using hibiscus by the green synthesis method could penetrate the cell wall and effectively kill bacteria present in polluted water by agar well diffusion assay. Silver nanoparticles can interact with bacterial cell membranes, causing structural damage in the cell wall. This disrupts the cell membrane's integrity, leading to cellular contents leakage and cell death [28]-[29]. Four bacterial strains usually exist in contaminated water samples: *Pseudomonas, Acinetobacter, Cholera,* and *Escherichia coli*; the antibacterial activity of the synthesized Ag NPs is shown in Figures 7, 8, 9, and 10. As indicated in the results, the antibacterial activity increases by increasing the concentration of Ag NPs due to an increase in the particles destroying bacteria cell membranes. Indeed, certain limits must not be reached to avoid the toxicity effect of silver. [30]. It was revealed through Ag NP annotation that the Ag NPs cause varying inhibition zones against the investigated pathogens. Against *Acinetobacter*, the maximum zone of inhibition up to 25 mm was found, followed by *Pseudomonas* up to 23 mm, *Cholera* up to 16 mm, and *Escherichia* coli up to 15 mm. Table 5 shows the inhibition values at different concentrations.

Table 5. Antibacterial activity of Ag NPs

Antibacterial analysis (Zone of inhibition (mm))					
Sample	Control	12.5%	25%	50%	100%
Pseudomonas	6	10	13	15	23
E.coli	6	7	7	8	15
Acinetobacter	6	20	22	24	25
Cholera	6	12	12	13	16

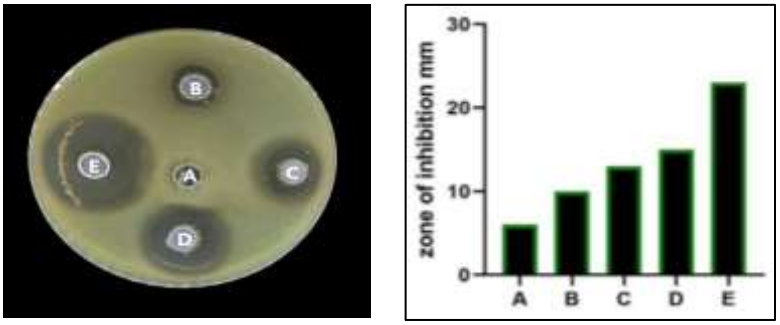

Figure 7. Antibacterial activity of Ag NPs against *Pseudomonas*: (A) Control (B) 12.5% (C) 25% (D) 50% (E) 100%

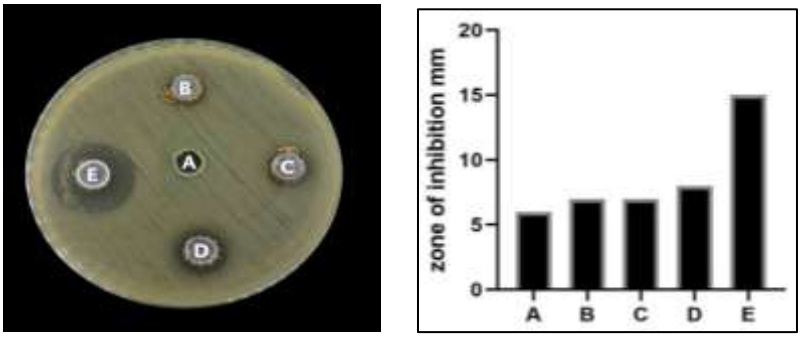

Figure 8. Antibacterial activity of Ag NPs against *E. coli*: (A) Control (B) 12.5% (C) 25% (D) 50% (E) 100%

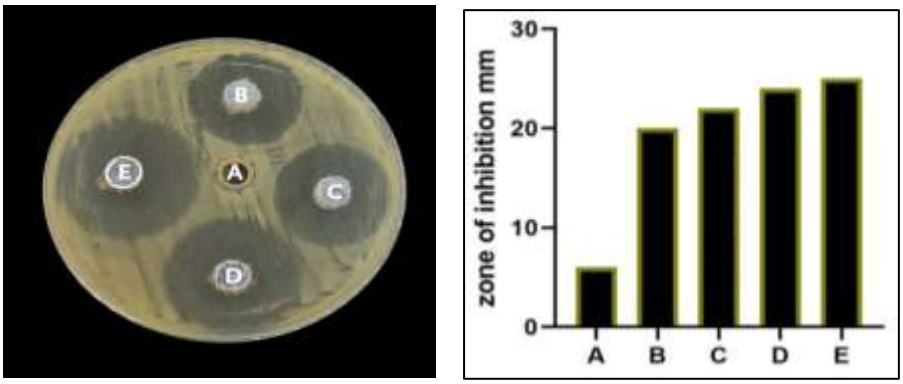

Figure 9. Antibacterial activity of Ag NPs against *Acinetobacter*: (A) Control (B) 12.5% (C) 25% (D) 50% (E) 100%

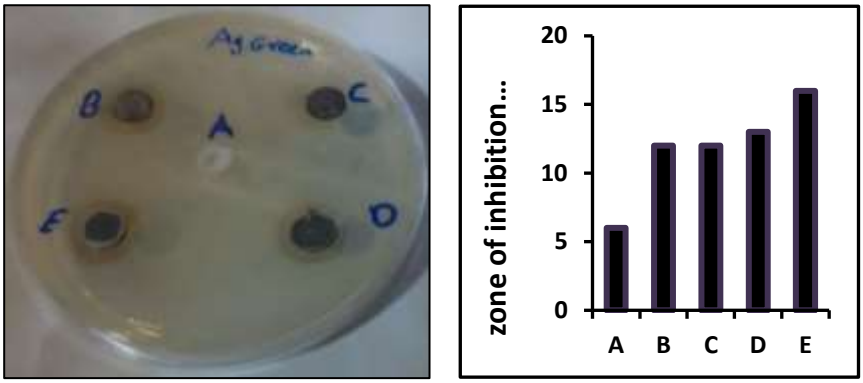

Figure 10. Antibacterial activity of Ag NPs against *Cholera*: (A) Control (B) 12.5% (C) 25% (D) 50% (E) 100%

4. CONCLUSION

The treatment successfully decreased the levels of TDS, TSS, and the total count of aerobic bacteria, demonstrating the antibacterial properties of the silver nanoparticles against pathogens commonly found in contaminated water. This method offers a promising approach to improving water quality in polluted rivers.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

SOURCE OF FUNDING

Self-funded

ETHICAL CLEARANCE

Ethical approval was not required for this study as it did not involve human or animal subjects.

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