

Evaluation of The Efficiency of Green-synthesized Silver Nanoparticles using *Salix babylonica* Leaf Extract for PAHs Remediation

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ABSTRACT

Objective: The study was conducted in the laboratories of the College of Science at Al-Qadisiyah University. Its aim was to evaluate the efficiency of silver nanoparticles (AgNPs) manufactured using a green process with an aqueous extract of willow leaves (*Salix babylonica*) and their use in the non-biological treatment and removal of polycyclic aromatic hydrocarbons (PAHs) from contaminated soil. These compounds are highly toxic and difficult to remove, posing a significant threat to the environment. The study included an evaluation of the removal efficiency of Naphthalene, Acenaphthene, and Pyrene. **Method:** The prepared nanoparticles were characterized using UV-Vis, XRD, SEM, and FTIR techniques, which confirmed the successful reduction of silver ions and the formation of highly stable spherical nanoparticles. The experiments involved treating industrially contaminated soil with different concentrations of silver nanoparticles (0.1, 0.01, and 0.001 mg/L), and the treatment was monitored for a period of 7 days. **Results:** The treatment results showed significant variation in removal rates depending on the nanoparticle concentration, with the highest removal rates of PAHs being recorded at a concentration of 0.1 mg/L. **Novelty:** The study concludes that the green manufacturing of silver nanoparticles using willow leaf extract represents an economical, efficient and environmentally friendly technique for treating soils contaminated with PAHs while preserving and improving soil properties.

INTRODUCTION

Soil pollution by polycyclic aromatic hydrocarbons (PAHs) is one of the most serious contemporary environmental challenges, as they are persistent organic pollutants that resist degradation and have the ability to accumulate and persist in the environment for long periods.[1] These compounds originate from natural processes and human activities such as the incomplete combustion of organic materials, industrial activities, oil refining, and accidental oil spills.[2] Many PAHs have been proven to have carcinogenic, mutagenic, and teratogenic properties.[3] Therefore, their accumulation in the soil leads to the deterioration of the physicochemical properties of the environment and poses a significant threat to the food chain, human health, and other living organisms [4].

Recent years have witnessed numerous strategies for addressing environmental pollution, with nanotechnology emerging as a promising and innovative solution for treating environmental pollutants (nanoremediation) [5]. Silver nanoparticles (AgNPs) are among the most efficient and effective nanomaterials due to their unique properties, such as their high specific surface area and superior chemical activity [6]. However, traditional (chemical and physical) methods for manufacturing these particles often use toxic and energy-intensive chemical compounds, prompting researchers to seek a

sustainable and safe alternative: the green manufacturing of nanoparticles using living organisms or plant extracts as environmentally friendly reducing and stabilizing agents [7].

In this context, the willow plant (*Salix babylonica*) stands out as an ideal biofilter because its leaf extract contains high concentrations of biologically active compounds such as phenolic compounds and flavonoids, which are extremely and effectively able to reduce silver ions to their stable nano-form with high efficiency, in an economical, safe and non-toxic manner [8].

This study aims to biosynthesize silver nanoparticles using an aqueous extract of willow leaves as a natural reducing agent, determine their physicochemical properties, and evaluate their efficiency and analytical capacity in removing three types of targeted polycyclic aromatic hydrocarbons (PAHs) from contaminated soil.

RESEARCH METHOD

Soil preparation and laboratory contamination with PAHs

Soil samples were collected from an agricultural field in Al-Diwaniyah Governorate from a depth of (0-20) cm and placed in sterilized containers. Visible impurities were removed, and then the soil was sieved using a sieve with 2 mm diameter openings to ensure homogeneity. The soil was air-dried at room temperature for 24 hours, after which it was sterilized using an autoclave at a temperature of 121°C for 20 minutes for two consecutive days to completely eliminate heat-resistant spores and microorganisms. The soil was left to cool in a well-ventilated place and was stored in tightly sealed, sterilized containers in a dark, dry place [9]. To artificially contaminate soil with PAHs (Naphthalene, Acenaphthene, Pyrene) to achieve a typical concentration of 500 mg PAHs per kg of soil [10], the contamination process involved dissolving 100 mg of the contaminants in 100 ml of hexane solvent to obtain a homogeneous solution. This solution was then gradually added to 200 g of soil while continuously mixing to ensure uniform distribution of the contaminants within the soil. The contaminated soil mixture was placed inside a laboratory aeration system (fume hood) with daily stirring to allow the soil to dry completely and the organic solvent to evaporate before treatment began.

Green synthesis of silver nanoparticles (AgNPs) from willow leaf extract

The aqueous extract of willow leaves (*Salix babylonica*) was prepared according to protocols from previous studies with some minor improvements [11]. The willow leaves were first washed with ordinary water to remove dirt and impurities, then washed repeatedly with deionized water. They were then air-dried at room temperature until a stable mass was reached, and finally ground into a fine powder. 15 g of the powder were taken and 100 ml of deionized water were added to it in a sterile glass flask. The mixture was heated in a water bath at 70-80°C, stirring continuously for 30 minutes without bringing it to a boil. The mixture was then purified using Whatman No. 1 filter paper and centrifuged at 8000 rpm for 15 minutes to obtain the clear aqueous extract.

AgNPs were prepared using the green synthesis method. 5 ml of aqueous willow leaf extract were gradually added to 50 ml of 1 mM silver nitrate (AgNO_3) solution in a sterile glass flask. The pH was adjusted to the desired value (8), and the mixture was heated to 80°C for 30 minutes with continuous stirring. The solution gradually changed color from colorless to dark brown, indicating the formation of silver nanoparticles resulting from the reduction of silver ions (Ag^+) to metallic silver (Ag^0) by the bioactive compounds present in the plant extracts. After the synthesis reaction was complete, the colloidal suspension was allowed to cool gradually to room temperature and then stored in tightly sealed, sterile, opaque glass bottles at a low temperature until use in subsequent experiments.

Characterization of the properties of silver nanoparticles:

The physical and chemical properties of the silver nanoparticles synthesized using the aqueous extract of willow leaves were characterized using a range of advanced analytical instruments and techniques, including ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR), in order to prove the formation of the nanoparticles and characterize their structural and morphological properties.

Structure of the treatment and pollutant extraction experiment

The experimental design employed three concentrations per treatment and three replicates per concentration to ensure accurate results. A colloidal suspension of silver nanoparticles was used as a standard solution at an initial concentration of 500 mg/L. Dilution was carried out using deionized water under sterile laboratory conditions to prepare three different concentrations: 0.1, 0.01, and 0.001 mg/L. One hundred grams of contaminated soil were weighed and placed in suitable, sterile containers. 10 ml of one of the prepared silver nanoparticle concentrations were added to each soil sample, with thorough mixing to ensure homogeneous distribution of the colloidal suspension within the sample. Soil moisture levels were adjusted to approximate field capacity using deionized water when necessary. Treated soil samples and control (untreated contaminated soil) were incubated at room temperature for 7 days, with periodic stirring to ensure aeration and homogeneity of the reaction.

For extraction, 10 g of each soil sample was taken and 10 mL of hexane solvent was added. The samples were shaken mechanically for 60 minutes to ensure the extraction of PAHs. The extraction process was repeated twice under the same conditions to guarantee efficiency. The filtrate was then separated using Whatman No. 2 filter paper and filtered a second time using a filter connected to a syringe with $0.22\ \mu\text{m}$ apertures to obtain a pure extract free of suspended particles. The extract was then concentrated using a rotary evaporator to a final volume of 5 mL and stored in dark glass bottles at 4°C until GC-MS analysis.

Quantitative analysis using GC-MS technology:

The determination of PAHs residues in soil extracts was performed using a Shimadzu GC-MS 2010 gas chromatography-mass spectrometry (GC-MS) system of

Japanese origin. The system is equipped with an AOC-20i+s automatic identification unit for the compounds. A 1 μ L sample was taken and injected into the system, and the ion source and interface temperatures were set to 250°C and 230°C, respectively.

The column furnace temperature was set at 70°C at the start of operation with a holding time of 2 minutes, then the temperature was increased at a rate of 10°C/minute to reach 290°C with a final holding time of 15 minutes. The compounds were identified based on the interpretation of the GC-MS mass spectra and retention times, and the results were matched with the National Institute of Standards and Technology (NIST) global database, which contains more than 62,000 known spectral patterns, to accurately confirm the name, molecular weights, and molecular structure of the compounds measured by the instrument. The extent of their reduction and biodegradation efficiency was assessed by calculating the percentage of biodegradation (%) $C_0 - C_t / C_0 \times 100$ based on the results of the GC-MS analysis. These values were used to compare the effectiveness of different treatments in reducing the concentration of petroleum hydrocarbon pollutants in the soil.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles (AgNPs) from willow leaf extract

UV-Vis spectroscopy of silver nanoparticles prepared using willow leaf extract

In the UV-Vis spectrum of Ag NPs biosynthesized by using the *Salix* extract, a distinct SPR band is evident, ranging from 410 nm as shown in figure (1). The SPR band is one of the optical signatures frequently used to detect biosynthesized Ag NPs. The SPR band is evident over a relatively shorter wavelength, which is a hint of smaller particle size and less aggregation of the prepared Ag NPs biosynthesized using plant-based methods. The broad SPR band is another characteristic of agglomerated nanoparticles, which is typical of biosynthesized Ag NPs. A higher UV absorption band is typical of the presence of phenolic and flavonoid compounds of the plant extract and is associated with $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of phenolic compounds and other agro-based materials. The decrease in the intensity of absorption over a longer wavelength is a characteristic of the presence of nanoparticles, which is a clear hint of stable Ag NPs biosynthesized using plant-based materials [12].

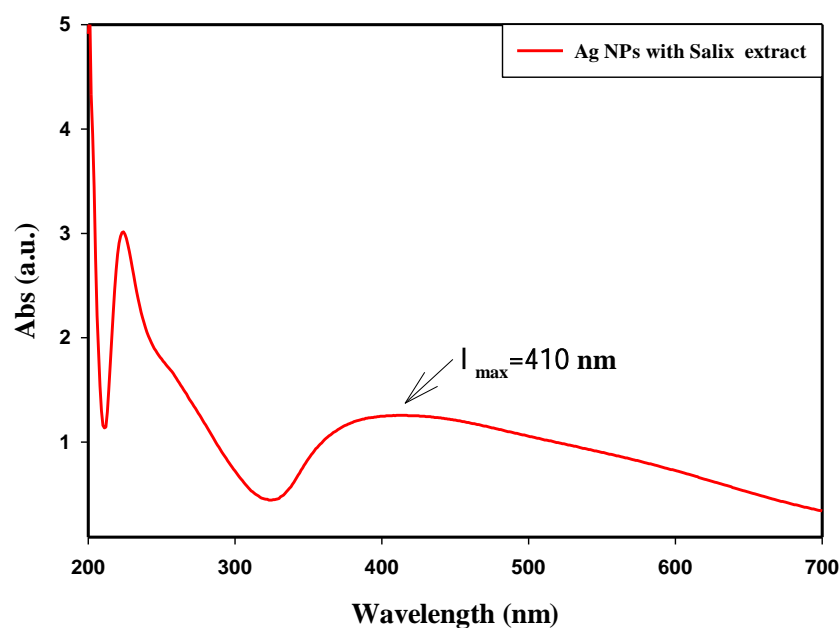


Figure 1. UV-Vis spectrum of Ag NPs prepared by Salix extract

X-ray diffraction (XRD) pattern of silver nanoparticles prepared from willow leaf extract

Figure (2) shows the X-ray diffraction (XRD) analysis of silver nanoparticles synthesized using Salix (willow) extract displayed four characteristic diffraction peaks at $2\theta = 38.44^\circ, 44.50^\circ, 64.84^\circ,$ and 78.04° , which correspond to the (111), (200), (220), and (311) planes of face-centered cubic (FCC) silver, respectively (Table 1). The most intense peak of this test at the (111) plane indicates a preferred orientation along this crystallographic plane. Crystallite sizes calculated by the Scherrer equation range from 15 to 22 nm, indicating the formation of nanoparticles at the nanoscale [13]. The sharpness and clarity of the peaks suggest good crystallinity and purity, highlighting the efficiency of Salix biomolecules as natural reducing and stabilizing agents in the green synthesis process.

Table 1. XRD result of silver nanoparticles synthesized by Salix extract.

2θ ($^\circ$)	d (nm)	FWHM ($^\circ$)	Crystal Size XS (nm)	Miller Indices (hkl)
38.443	0.23397	0.571	15	(111)
44.495	0.20345	0.547	16	(200)
64.844	0.14367	0.430	22	(220)
78.042	0.12234	0.537	19	(311)

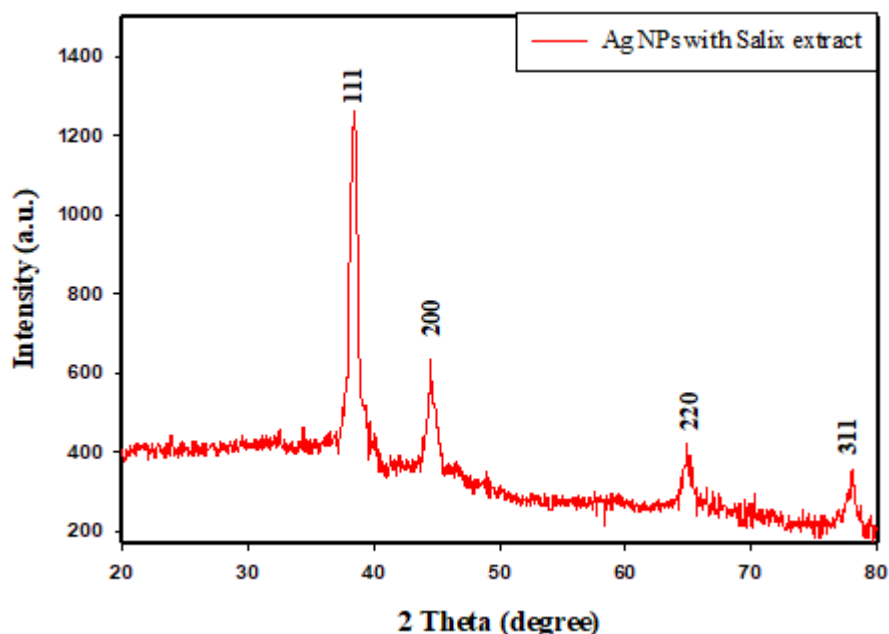


Figure 2. XRD of silver nanoparticles synthesized by Salix extract

AgNPs of SEM prepared using willow leaf extract

SEM images of nanoparticles prepared using willow leaf extract (Figure 3) showed the formation of relatively irregular, quasi-spherical nanoparticles with distinct agglomerations, a rough surface texture and heterogeneous distribution in some areas. The measured nanoparticle diameters ranged from 14.5 to 58.0 nm, with an average particle size of 36.8 nm. This relatively wide size range reflects variations in particle growth during preparation, a common pattern when using plant extracts rich in phenolic compounds that act as both reducing and stabilizing agents, but which may promote agglomeration. These results are consistent with previous studies that used plants of the genus *Salix* in the green synthesis of silver nanoparticles, where particles within the nanometer range were recorded with the appearance of clear surface clumps resulting from the nature of the bonds between the particles and the surrounding organic materials [14] [15].

The use of willow resulted in particles with higher agglomeration and less homogeneity. Previous studies on the green synthesis of nanoparticles have indicated that the nature of the biocomposites and the preparation conditions critically control the morphological and size characteristics of the particles [16]. These morphological characteristics are of great importance in interpreting the environmental performance of nanoparticles, particularly in applications for removing complex organic pollutants from soil.

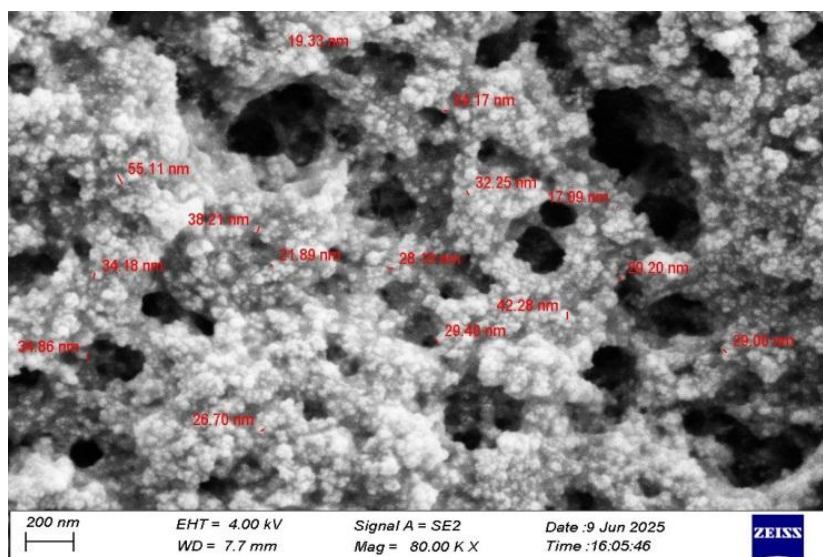


Figure 3. Scanning electron microscope (SEM) images of silver nanoparticles prepared from willow leaf extract at a magnification of (x80000) and with a 200 nm bar.

Willow extract 3-1-4 FTIR of AgNPs

The FTIR spectrum of silver nanoparticles synthesized using willow extract indicates that different bioactive functional groups are responsible for the reduction and stabilization of the nanoparticles (Figure 4). The broad band observed between 3416 cm^{-1} and 3240 cm^{-1} is attributed to the O-H tension vibrations of the bioactive phenolic and flavonoid compounds of willow, along with the N-H vibrations of the proteins.

The absorption bands at 2916 and 2849 cm^{-1} correspond to the aliphatic C-H tension. The absorption bands observed at 1636 and 1616 cm^{-1} correspond to the carbonyl and C=C aromatic tension of salicylic acid and its derivatives. The absorption band observed at 1557 cm^{-1} corresponds to the amide II band of proteins. The absorption bands in the region from 1383 to 1043 cm^{-1} correspond to the carboxyl and phenolic C-O tension. The absorption bands in the low wavenumber region below 600 cm^{-1} correspond to the Ag-O tension. These peaks indicate the successful preparation of silver nanoparticles using an environmentally friendly bioassay [17].

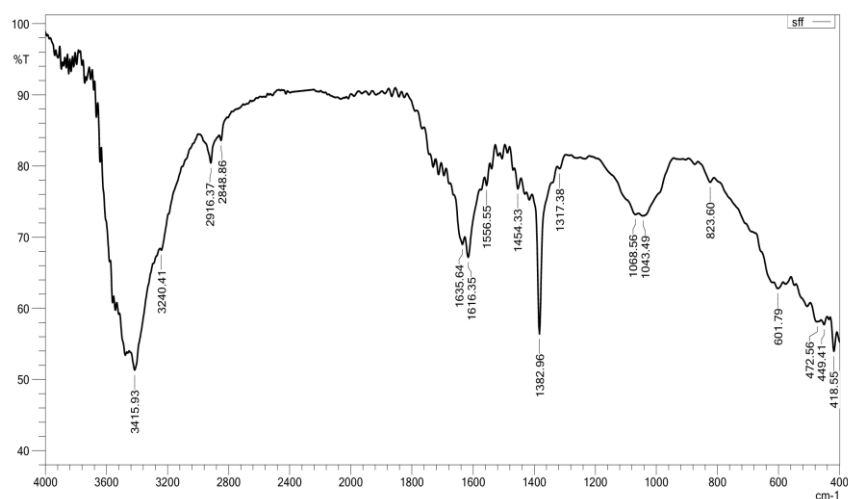


Figure 4. FTIR of Ag NPs prepared using willow extract

Results of analysis of soil samples contaminated with PAHs after 7 days of treatment

Table (2) shows the results of the effect of silver nanoparticles prepared from willow leaf extract on removing polycyclic aromatic hydrocarbons (PAHs) from contaminated soil, including naphthalene, acenaphthene, and pyrene. The results showed high efficiency, leading to a decrease in the concentrations of these compounds after 7 days of treatment, as analyzed by GC-MS, compared to the control treatment. The results indicate a direct relationship between increasing the concentration of willow extract and the efficiency of PAH removal.

Table 2. Effect of silver nanoparticles of willow leaf extract at different concentrations (mg/L) on the concentration and percentage of PAH removal in the soil after 7 days of treatment using GC-MS technology

Concentration compound	Control (mg/L)	Con. 0.001 (mg/L)	Con. 0.01 (mg/L)	Con. 0.1 (mg/L)	LSD P<0.05
		SD± mean R.%	SD± mean R.%	SD± mean R.%	
Naphthalene	15.48	0.01± 3.91	0.020± 1.89	0.000 ± 0	0.025
		74.74%	87.79 %	100 %	0.918
Acenaphthene	12.43	0.04± 5.1	0.02±3.16	0.015 ± 1.98	0.056
		59.00%	74.60%	84.10%	1.095
Pyrene	13.06	0.02± 6.34	0.02±4.23	0.02 ±2.39	0.063
		51.00%	67.60%	81.70%	1.112

SD ± mean values represent the mean ± standard deviation of the residual concentration of PAHs (mg/L) and R.%. The percentage removal of the compound and LSD (P<0.05) represent the least significant difference at a 5% probability level

For naphthalene, the concentration decreased at all nanoparticle concentrations used, reaching 3.91, 1.89, and 0.00 mg/L at concentrations of 0.001, 0.01, and 0.1 mg/L, respectively, compared to the control treatment (15.48 mg/L), with removal percentages of 74.74%, 87.79%, and 100%. The results, with a least significant difference (LSD = 0.025 for concentration and 0.918 for removal percentage), indicate that the differences between the treatments and the control treatment were significant, suggesting the high efficiency of the nanoparticles in removing pollutants, especially at the higher concentration.

As for Acenaphthene, it approached 5.1, 3.16 and 1.98 mg/L compared to a control factor of 12.43 mg/L and with clearance rates of 59.00%, 74.60% and 84.10% on the toilet, compared to the value of the least difference with 0.056 LSD for concentration and 1.095 L clearance rate. Significant differences were considered between the different values and

different concentrations, indicating a high degree of variability that remains defined with increasing intensity of variability.

While Pyrene showed the lowest removal rates compared to Naphthalene and Acenaphthene, with removal rates of 51.00%, 67.60% and 81.70% at concentrations of 0.001, 0.01 and 0.1 mg/L respectively, compared to the control treatment of 13.06 mg/L, LSD values of 0.063 for concentration and 1.112 for removal rate indicate significant differences between the different treatments and concentrations.

The high efficiency of bio-prepared silver nanoparticles in removing PAHs from contaminated soil is related to both the nanoparticle concentration and the nature of the biosource used in the greening process. Increasing the concentration from 0.001 to 0.1 mg/L resulted in a consistent increase in removal efficiency, culminating in complete removal (100%). This can be explained by the fact that increasing the nanoparticle concentration leads to an increase in the effective surface area and the number of active sites available for reaction, which enhances the efficiency of adsorption, degradation, or chemical transformation of the compound within the soil. These results are consistent with those of [18][19] in their study on the use of green-processed silver nanoparticles for PAH removal, where they confirmed that removal efficiency increases proportionally with increasing nanoparticle concentration.

Achieving 100% removal at a concentration of 0.1 mg/L confirms that increasing the adsorbent dose necessarily leads to increased pollutant removal efficiency. This is due to the availability of sufficient surface energy to break the bonds or completely adsorb the compound. This result aligns with the findings of [20] [21] regarding the effectiveness of bio-prepared silver nanoparticles for the complete removal of complex hydrocarbons. It also supports the findings of [22] regarding the importance of nanotechnology as a sustainable solution for removing toxic pollutants from the environment.

The ability of willow nanoparticles may be due to the nature of the phytochemical content of the extract, which acts as a more efficient capping agent, preventing particle aggregation and maintaining their high activity within the soil pores. This result is consistent with a study conducted by [23] , which showed the superiority of willow extract (*Salix babylonica*) in producing nanoparticles with high stability and great catalytic ability in environmental remediation, and that the nanoparticles manufactured by *Salix* (willow) extracts have distinct physicochemical properties that give them a high catalytic ability in treating pollutants that surpasses traditional chemical methods.

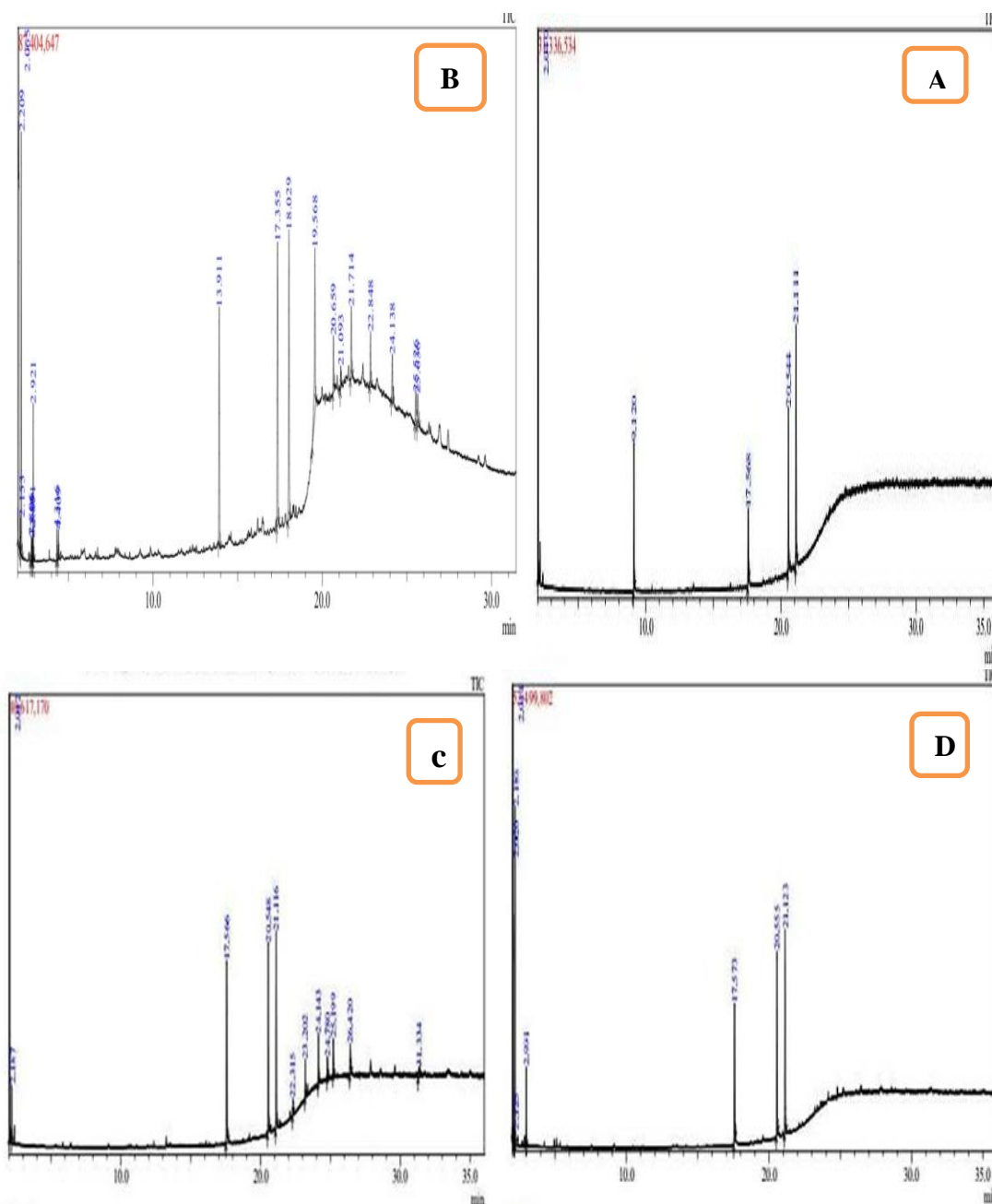


Figure 5. Gas chromatography-mass spectrometry (GC-MS) plots showing the efficiency of bioprepared silver nanoparticles using willow leaf extract at different concentrations for removing PAHs from contaminated soil. (A) Control treatment (B) 0.1 mg/L concentration (C) 0.01 mg/L concentration (D) 0.001 mg/L concentration

CONCLUSION

Fundamental Finding: The study succeeded in synthesizing highly efficient silver nanoparticles (AgNPs) in an environmentally friendly bio-method using the aqueous extract of willow leaves. The results of the structural characterization using (UV-Vis, XRD, SEM, FTIR) techniques confirmed the formation of silver nanoparticles with semi-spherical shapes, high crystalline purity, and an average particle size of 36.8 nanometers. The phenolic compounds and proteins present in the willow leaf extract contributed to the high stability of the particles and prevented their aggregation, as they played an

important role as reducing and encapsulating agents. The results of the bioremediation showed the high efficiency of silver nanoparticles from willow leaves in removing PAHs (naphthalene, acenaphthene, pyrene) from laboratory-contaminated soils. GC-MS analysis of the contaminated soil samples indicated that the treatment led to very high rates of pollutant degradation, reaching 100% for some compounds after a treatment period of 7 days. **Implication:** The results also showed a direct relationship between the percentage of pollutant removal and the concentration of nanoparticles, with a concentration of 0.1 mg/L yielding the highest rate of compound removal compared to concentrations of 0.01 and 0.001 mg/L. The study concludes that the use of bio-prepared silver nanoparticles from willow leaf extract possesses highly efficient and stable physical and chemical properties in environmental remediation techniques, in addition to being an eco-friendly technology, making it an ideal and sustainable option for eliminating soil pollution with PAHs and improving soil properties. **Limitation:** The results of the bioremediation showed the high efficiency of silver nanoparticles from willow leaves in removing PAHs (naphthalene, acenaphthene, pyrene) from laboratory-contaminated soils. **Future Research:** The experiments involved treating industrially contaminated soil with different concentrations of silver nanoparticles (0.1, 0.01, and 0.001 mg/L), and the treatment was monitored for a period of 7 days.

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