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TEM and XAS Study of Silver Nanoparticles Formed in *Phaseolus vulgaris*

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Research Article

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ABSTRACT

The phytoremediation can be used to recover precious metals like gold, silver, platinum and palladium, indicating that there is a wide opportunity for use it as a remediation technology in mining. The alfalfa alive was used to reduce the silver and forming silver nanoparticles Ag (0). The plant phaseolus vulgaris (beans) was used to form silver nanoparticles through bioreduction of Ag (I) to Ag (0) in the living plant. Two groups of plants were used. One group of plants grew at garden soil and the other in cotton. In both cases, a solution of $AgNO_3$ was added initially in a concentration of 0.01M then the concentration was changed to 0.1mm. The samples were analyzed with X-Ray Absorption Spectroscopy (XAS) at the Stanford Synchrotron Radiation Laboratory (SSRL) and later with Transmission Electron Microscopy (TEM). Analyzing the media of cultivation in soil and cotton and also in the roots of the plants, silver was found as silver oxide (AgO). In stem and leaves silver was found as Ag (0). The XAS spectra were adjusted for more accurate results. The plant has the ability to reduce the valence of silver and form nanoparticles. The TEM images show that the average particle size is 18 nm, showing in various forms and a greater number of them in the leaves of plants grown in soil.

Keywords: Phaseolus vulgaris, silver nanoparticles, XAS, XANES, phytoremediation, $AgNO_3$.

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1. INTRODUCTION

Within the area of nanotechnology, the preparation of metal nanoparticles gained great interest recently due to the peculiarities of their optical, magnetic, electrical and catalytic properties. Many of these properties and their potential applications are heavily influenced by the size and shape of these: spheres, rods, discs, prisms, etc.

Silver metal in bulk or finely dispersed, is a material becoming most important in many technologies. It exhibits unique features normally associated with the noble metals (chemical stability, excellent electrical conductivity, catalytic activity, etc.), together with others more specific such as bacteriostatic effects, nonlinear optical behavior, etc. (Roldán et al., 2000).

This is why in recent times various techniques for preparation of nanoparticles to control the morphology of the product have been developed. Recently, the use of biological systems has emerged as a new method for the synthesis of nanoparticles (Peralt, 2002). The uptake of silver and the formation of silver nanostructures inside live alfalfa plants have been investigated. Besides, the Verticillium fungus isolated from the plant Taxus facilitates an intracellular reduction of ions in aqueous solutions to produce silver nanoparticles of 25 $\pm$ 12 nm (Gardea, 2003; Chen et al., 2003).

In order to determine whether the plants have captured the silver, they have been analyzed by using X-ray absorption spectroscopy (XAS) such as it was tested in the case of the alfalfa (Duarte M. J. A., 1996). The XAS spectrum consists of two components: Extended X-ray absorption fine structure (EXAFS) and structure of X-ray absorption near the threshold (XANES) (Gardea et al., 2004). XANES provides information about the state of oxidation of silver in bean plants and in the growth medium, and EXAFS studies the neighboring of Ag atoms.

In this work, we used the *Phaseolus vulgaris* plant to extract silver from two different culture media and form silver nanoparticles within it.

American Pinto type seeds were tested within two different culture media that was cotton and garden soil. The plants were at a natural environment and a silver nitrate solution ($AgNO_3$) and water were added for growing. The samples were analyzed in transmission electron microscopy (TEM) and in XAS. With these, it was shown that bean plants might form silver nanoparticles of different sizes in both culture media. According to the literature, the bean plant has not been used for the formation of silver nanoparticles.

2. MATERIALS AND METHODS

Twenty bean seeds were planted in natural environment in two different culture media: garden soil and cotton. The plants were allowed to grow for two weeks watering only with pure water during that time. After this time, a solution of silver nitrate 0.01 M was added and after that, a solution of 0.1mM was also added. This solution was added for two weeks each 3 days and observing the reaction of plants to the solution.

When the bean plants in both culture media began to decrease their growth and began to turn to yellow color, the solution adding was stopped and then only water was added for 2 weeks more. After this, not more growth was seen neither a change in yellow color. Therefore, plants were left to dry.

2.1 XAS analysis

XAS is a technique that has been used in recent years to examine the structure of the surroundings of a selected atom. To prepare the sample once the plant is dry, the sample was washed three times with deionized water and immersed in liquid nitrogen for 40 minutes (Gardea et al., 2002).

Then, the samples were placed in a dryer for 2 days for dehydration and eventually grind biomass in a homogeneous fine powder using a mortar. Samples were taken to the Stanford Synchrotron Radiation Laboratory (SSRL) for XAS, including XANES and EXAFS of the Ag-K edge (25.514 keV). This analysis was conducted to investigate the oxidation state, the interatomic distances, and the number of the nearest neighbor atoms to silver atoms in different parts of plants. The condition was a typical operating current of 60 - 100 mA and energy of 3 GeV. All samples were run at a temperature near 15 K using a helium cryostat to reduce the effects of Debye-Waller thermal disorder presenting in samples. The fluorescence spectra of the samples were taken with the help of a Canberra germanium detector of 30 elements.

2.2 TEM Analysis

The biomass obtained was washed with deionized water and dried in an oven. The dry biomass was grinded and sieved to get particles of uniform size. Before the implementation of the experiment, biomass was filtered, washed twice with 0.01M HCl and also washed twice with deionized water to remove any material that could interfere with the link and the nanoparticles formation of Ag (0) (Gardea et al., 2003). The samples were analyzed using a microscope JEOL 4000 EX high-resolution following the same procedure previously described for the formation of gold nanoparticles by alfalfa biomass (Gardea et al., 2004).

To analyze the XAS spectra of the samples, the WinXAS software was used. This is a program for XAS data analysis. WinXAS represents a useful tool to analyze atomic distances and coordination numbers through χ(k) (Torstein, 1999-2004).

Energy in spectra was calibrated with the 1st and 2nd derivative of E_0 and determining had a value of 25.3 KeV. Background was corrected by two polynomials of 1st grade. Spectrum was normalized to 1 by a polynomial of order zero and made the conversion of the spectrum from E to k vector for space domain R and an adjustment of μ (0) by cubic spline (polynomial of third order) to choose for Fourier transform which shows us the Radial Distribution Function characteristic of the element under study and provides data for the number of neighboring atoms to the silver atom and the distance to the scattered atom.

3. RESULTS AND DISCUSSION

XAS. Samples of soil, cotton and roots differ in the shape of the spectrum (Figure 1), these have a structure corresponding to Ag (II) indicating that there was no reduction.

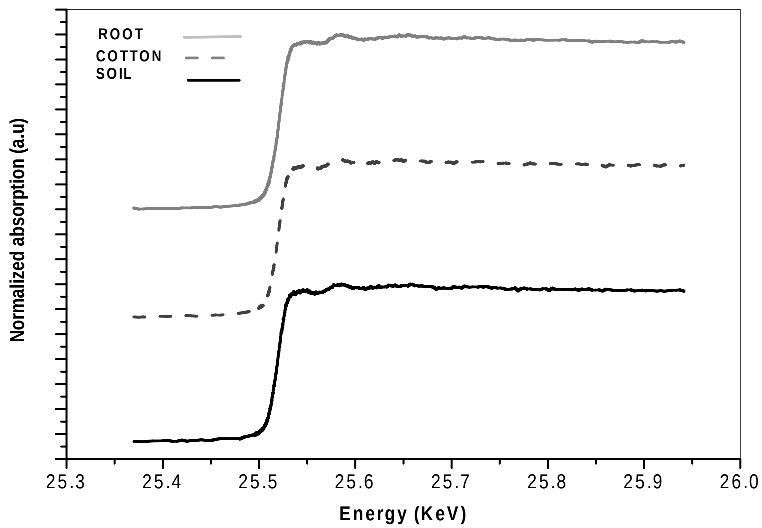

Fig. 1. Absorption spectra of culture media, (soil and cotton) and roots of plants

The following figures shown the whole spectrum of XAS around the ionization threshold k for the silver, located at 25.52 and 25.53 keV for each of the samples under study. In this region, it is possible to establish the type or chemical valence of the element in question, namely Ag. Figure 2 and 3 shows that there was a reduction in the valence of silver and consequently, the formation of nanoparticles.

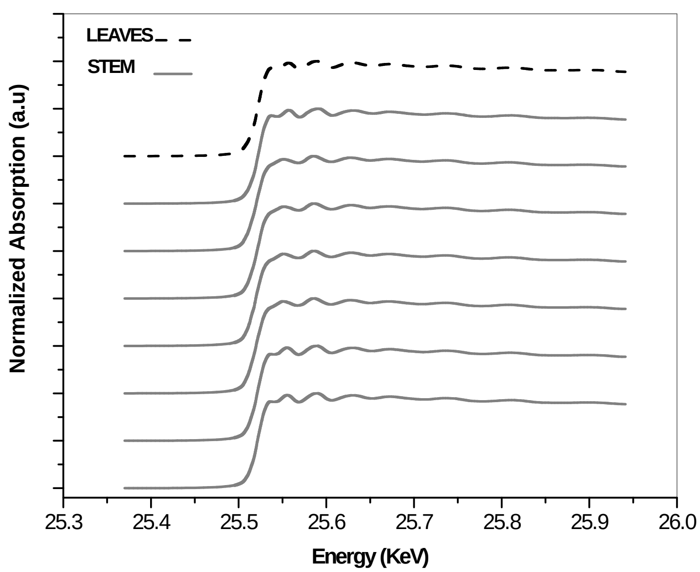

Fig. 2. Absorption spectra of stem and leaves samples which grew in cotton

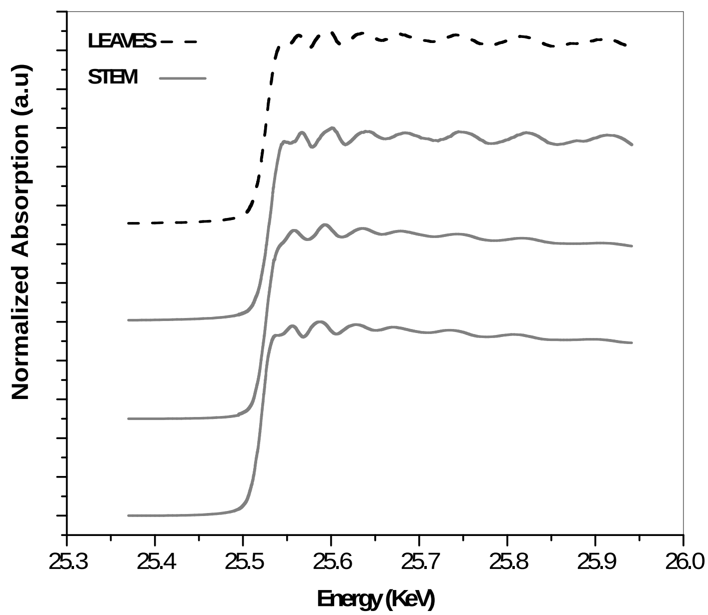

Fig. 3. Absorption spectra of stem and leaves samples which grew in soil

3.1 Fast Fourier Transform

Figure 4, 5 and 6 present the RDF (Radial Distribution Function) for each sample by applying the FFT to the spectra of Figures 1, 2 and 3 after a background correction and conversion to vector k by the relationship:

$$k = \sqrt{\frac{2m(E - Eo)}{\hbar^2}} \tag{1}$$

In there, dominant peaks are shown, corresponding to the inter-atomic distance. Figure 4 shows the respective Ag (II) and Figures 5 and 6 shows the respective Ag (0).

In Figure 4 the dominant peaks correspond to a distance between 0.133 and 0.192 nm. Which are characteristic of Ag-O link. Figures 5 and 6 show a gap between 0.2648 nm and 0.2673 nm, corresponding to the Ag-Ag bond.

3.2 EXAFS Fit

FEFF generates a theoretical model for the structure of silver and uses the parameters: N, R, σ^2 and ΔE, which is necessary data to generate the Radial Distribution Function (RDF) of the atoms that give us the number of neighboring atoms of the element and also the interatomic distance between them. For this research, we use the data for Ag and AgO.

The software uses these parameter to generate the $\chi(\kappa)$ and a Fast Fourier Transform (FFT) is applied to generate the theoretical RDF of Ag and AgO.

We chose the K edge and distances of neighboring atoms that we want to adjust to our experimental spectra and set the coordination number and σ^2. The program adjusts the theoretical FFT to the experimental FFT and fits it too. And so, we have the adjustments.

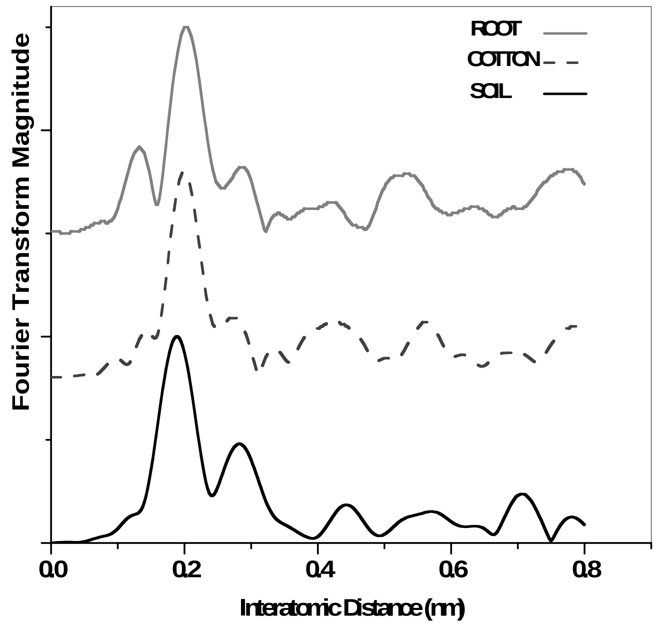

Fig. 4. Fourier transform of culture media (cotton and soil) and roots.

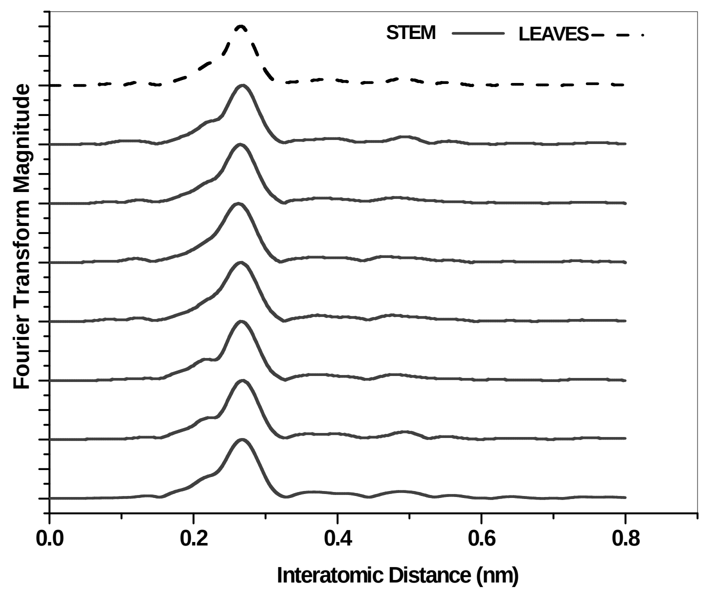

Fig. 5. Fourier transform of leaves and stems of plants grown in cotton.

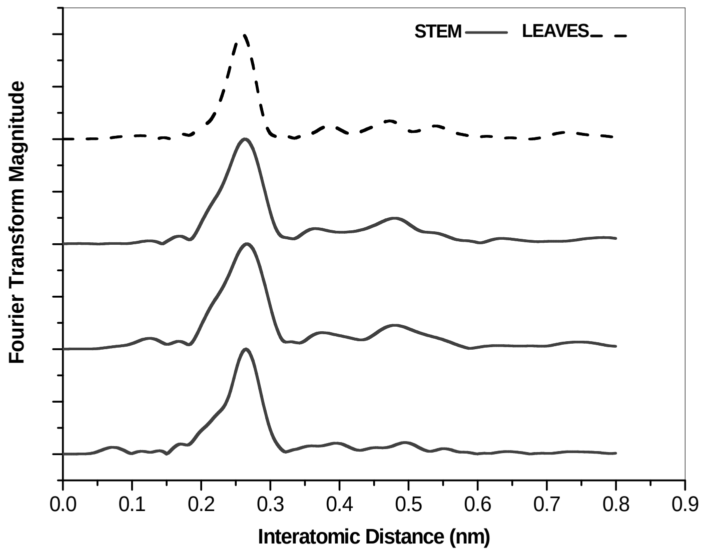

Fig. 6. Fourier transform of leaves and stems of plants grown in soil

Tables 1 and 2 shows the results obtained through adjustments framed primarily in two models: the metallic silver and silver oxide.

Table 1. Energy calibration E_0, Interatomic Distance R (Å), Coordination Sumner N and ΔE in plant grew in cotton

Samples grew in Cotton	E_0 (KeV)	R_1 (nm)		R_2 (nm)		N_1	N_2	ΔE
		Exp	Fit	Exp	Fit			
Cotton	25.52	0.1430 **Ag-O**	0.2316	0.2008	0.2636	1.854	2.04	7.76 ± 0.039
Stem	25.52	0.2680 **Ag-Ag**	0.2898	-	0.4067	13.661	6	4.688
Stem	25.5	0.2678 **Ag-Ag**	0.2898	-	0.4141	13.661	6	4.541
Stem	25.52	0.2638 **Ag-Ag**	0.2899	-	0.4020	13.127	6	4.980
Stem	25.51	0.2631 **Ag-Ag**	0.2895	-	0.4143	13.258	6	4.202
Stem	25.52	0.2605 **Ag-Ag**	0.2893	-	0.4055	12.676	6	-0.032
Stem	25.52	0.2625 **Ag-Ag**	0.2900	-	0.4216	12.803	6	4.426
Stem	25.52	0.2645 **Ag-Ag**	0.2916	-	0.4080	12.487	6.21	6.370
Leaves	25.52	0.2638 **Ag-Ag**	0.2899	-	0.4092	12.239	8.33	4.916

Table 2. Energy calibration E_0, Interatomic Distance R (Å), Coordination Sumner N and ΔE in plant grew in garden soil

Samples grew in soil	E_0 (KeV)	R_1 (nm)		R_2 (nm)		N_1	N_2	ΔE
		Exp	Fit	Exp	Fit			
Soil	25.52	0.1921 **Ag-O**	0.2291	0.281	0.2738	2	2	0.125
Root	25.52	0.1335 **Ag-O**	0.2339		0.2664	1.65	2	0
Stem	25.52	0.2645 **Ag-Ag**	0.2923	-	0.4828	12.48	6.02	6.018
Stem	25.52	0.2625 **Ag-Ag**	0.2885	-	0.4103	12	6	2.80 ± 0.04
Stem	25.53	0.2598 **Ag-Ag**	0.2890	-	0.4181	12	6	-1.015
Leaves	25.53	0.2592 **Ag-Ag**	0.2887	-	0.4116	12	6	-3.36 ± 0. 46

3.3 TEM Results

The pictures below obtained by TEM show the silver nano-particles formed in stem and leaves. Figure 7a shows the Ag nanoparticles formed in the leaf of the plant grown in a culture medium of cotton. The average particle size was 18 nm. There can be seen that the particles have different shapes, being spherical and pyramidal.

Figure 7b displays the silver particles formed in leaves of plants grown in soil, which has spherical shape of columns and bars. The average size is 18 nm.

In Figure 7c appear the silver particles in stem with average size of 18.5 nm. Here can be seen that the quantity of nanoparticles in the stem is lower than those in the leaves.

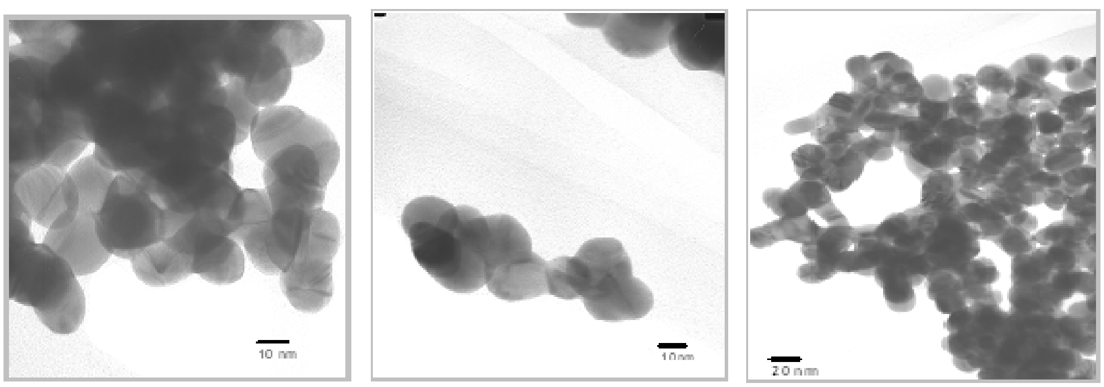

Fig. 7. a) Ag Nanoparticles in leaf, b) Ag Nanoparticles in leaves and c) Ag Nanoparticles in stem

The shapes of the nanoparticles depend of the plant's part where they were formed and the average size obtained is slightly higher in leaves than in stems. The pyramidal shape found in the stem is similar to those found in the leaves.

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4. CONCLUSION

The bean plants has the capacity to absorb the silver from a enriched culture medium which climbs up the stem and finally arrives to the leaves in the form of nanoparticles of Ag.

By XAS it is showed that silver nanoparticles are reduced to form Ag (0) in the stem and leaves and in the culture medium and the roots formed as AgO, because there is not still formation of nanoparticles.

In the TEM analysis, the images showed that were fewer Ag nanoparticles in the stem, and having a greater quantity in leaves, mainly in the plant that grew in a culture medium of garden soil. This is due to the nutrients that the plant can get for a better development. Nanoparticles obtained in the stem, leaves (cotton) and leaves (ground) have different forms and they can affect the soil pH.

Finally the average size of the nanoparticles in stem and leaves were 18 nm.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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