

## X-ray Absorption Spectroscopy Unveils the Formation of Gold Nanoparticles in Corn

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### ABSTRACT

In this study, X-ray absorption spectroscopy was used to determine the possible gold bio-transformation by *Zea mays* (corn) var. Golden, germinated and grown in a medium spiked with  $\text{KAuCl}_4$ . In addition, the gold uptake capacity of corn assisted by thiourea and ammonium thiocyanate was investigated. Results showed that up to 160 mg/L, gold did not reduce corn seed germination or plant growth. Both thiourea and ammonium thiocyanate resulted in a 6-fold increase of gold concentration in roots and thiourea promoted a 10-fold increase of gold concentration in shoots. X-ray absorption near edge structure studies demonstrated that approximately 91% of the gold present in plant samples was Au(0). The remaining 9% was present as Au(III). In addition, extended X-ray absorption fine structure results showed that in corn roots, the gold coordination number was around 9,5 neighboring gold atoms at approximately 2,86 Å, indicating an incomplete first coordination shell, which imply the presence of a nano-phase. The results demonstrated that *Z. mays* was able to produce gold nanoparticles with a size of 10,36 nm.

### RESUMEN

En este estudio se determinó, mediante espectroscopía de absorción de rayos-X, la posible biotransformación de oro en maíz (variedad Golden) que se germinó y creció en  $\text{KAuCl}_4$ . Adicionalmente se investigó el efecto de la tiourea y el tiocianato de amonio en la absorción de oro por la planta de maíz. Los resultados indicaron que concentraciones menores a 160 mg Au/L, no afectaron la germinación o el crecimiento de las plántulas. Tanto la tiourea como el tiocianato de amonio incrementaron 6 veces el contenido de oro en las raíces, mientras que la tiourea provocó un incremento de 10 veces la concentración de oro en tallos con respecto a los tratamientos sin este compuesto. El 91% del oro en el maíz se encontró como Au(0) y el resto como Au(III). Los análisis de estructura fina revelaron que el oro se encontraba con un número de coordinación de 9,5 aproximadamente a 2,86 Å, indicando una esfera de coordinación incompleta, lo cual implica la presencia de una nano-fase. Usando la ecuación de Borowski se determinó que las nanopartículas tenían un tamaño promedio de 10,36 nm.

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### INTRODUCTION

In recent years, the production of nanomaterials (those having at least one dimension smaller than 100 nm) has dramatically increased. Regarding precious metals, several researchers have reported the use of natural products, inactivated plant biomass, as well as living plants for the production of silver and gold nanoparticles (Gardea-Torresdey *et al.*, 2003; Lopez *et al.*, 2005; Rodriguez *et al.*, 2007; Philip, 2009). This strategy reduces the cost of production and the environmental impact. Plant species reported with the ability of forming gold nanoparticles include but are not limited to alfalfa (*Medicago sativa*), desert willow (*Chilopsis linearis*), and rattlebush *Sesbania drummondii* (Gardea-Torresdey *et al.*, 2002, 2003; Rodriguez *et al.*, 2007; Sharma *et al.*, 2007). In addition, the catalytic properties of gold nanoparticles produced by *Sesbania drummondii* seedlings have been demonstrated (Sharma *et al.*, 2007). Biotransformation is the chemical modification of a substance by means of living organisms or their metabolites (USGS,

#### Keywords:

*Zea mays*; Au; X-ray absorption spectroscopy; Gold nanoparticles; Thiourea; Ammonium Thiocyanate.

#### Palabras clave:

*Zea mays*; Au; Espectroscopía de absorción de rayos-X; Nanopartículas de oro; Tiourea, Tiocianato de amonio.

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2008). In this context, X-ray absorption spectroscopy (XAS) has been especially useful for the study of gold biotransformation in plant tissues (Gardea-Torresdey *et al.*, 2002).

In phytomining, metals of economic interest are recovered from ores or soils using plants (Anderson *et al.*, 1999a, 2005). For this purpose, metal hyperaccumulators are needed. In order to be considered a gold hyperaccumulator, a plant needs to be able to accumulate at least 1 mg per kg of dry biomass (Anderson *et al.*, 1999b; Brooks *et al.*, 1998), which is 100 times higher than gold concentrations normally found in plants.

Since gold uptake by plants is limited because of its low solubility under natural conditions, the use of chemical amendments for the enhancement of gold absorption has been explored. Anderson *et al.*, (1998) reported that *Brassica juncea* grown in a gold-containing medium amended with ammonium thiocyanate (AT) accumulated up to 57 mg Au kg<sup>-1</sup>, as compared to plants non treated with AT which extracted less than 0.1 mg of Au kg<sup>-1</sup>. In addition, Lamb *et al.*, (2001) showed that *Brassica juncea* and chicory increased gold uptake when they were added with cyanide. On the other hand, Msuya *et al.*, (2000) grew carrot, red beet, onion, and two cultivars of radish on gold containing media in the absence (controls) and presence of either ammonium thiosulfate (ATS) or AT. No gold was detected in controls; however, the presence of both compounds significantly increased gold content in some plant roots. Thiourea (TU) is another compound that has been used for gold solubilization as an alternative to cyanide (Hiskey, 1984). Some of the advantages of using TU is that it is less toxic (LD<sub>50</sub> in rat is 125 mg kg<sup>-1</sup>) than cyanide (LD<sub>50</sub> of NaCN in rats is 6,4 mg kg<sup>-1</sup>), which is commonly used to dissolve gold from ores (Deng *et al.*, 2001). In addition, TU acts as a growth promoter in some plant species. Khandelwal *et al.*, (2002) reported that flowers sprayed with TU at 2000 mg L<sup>-1</sup> augmented their weight and essential oil content. Sahu *et al.*, (1993) also reported an improvement of corn development. Regarding gold plant absorption, Rodriguez *et al.*, (2007) demonstrated that gold uptake by *Chilopsis linearis* roots increased more than 80% in the presence of TU, as compared to non-TU treated plants. Then, a whole picture would include the investigation of plant species suitable for gold uptake, their performance under treatments with chemical amendments, and their potential use for gold phytomining and nanoparticle production. The purpose of this research was to determine the capacity of corn for gold uptake from an agar-based media added with different concentrations of TU or AT, and to study the gold bio-

transformation inside plant tissues through the use of XAS. X-ray absorption near edge structure (XANES) was used to determine the oxidation state of gold and extended x-ray absorption fine edge structure (EXAFS) provided information about the nearest neighboring atoms for gold in the samples. Corn was selected since it is one of the crops grown worldwide, and because of its economic importance for some countries including Mexico. For this reason, this plant is convenient for testing its potential as a gold hyperaccumulator. The results will provide information on the toxicity of gold, TU, and AT in corn, as well as the ability of this plant to produce gold nanoparticles. This information can be used further to explore the possibility of using corn for phytomining and gold nanoparticle production.

## MATERIALS AND METHODS

### *Corn germination and growth in gold-agar media*

A modified Hoagland's nutrient solution (Peralta *et al.*, 2001) at pH 5,8 was used to prepare a 5% agar-based medium containing gold concentrations of 40, 80 and 160 mg L<sup>-1</sup> (from KAuCl<sub>4</sub>, Sigma, Aldrich). One-hundred and fifty mL of medium were poured on Mason jars, autoclaved for 35 min, and stabilized for one night. Later, 15 kernels of *Zea mays* (var. Golden) previously disinfected with a 15 % Captan solution were seeded on every Mason jar. Seeds were germinated in the dark for 4 days, and grown for 6 more days under a 14/10 h of light/dark cycle. Light for the experiment was provided by four 34 watts Philips lamps that produced an illumination of 39 mmol m<sup>-2</sup>s<sup>-1</sup>. The temperature during the whole experiment was of 25°C.

### *Effect of thiourea and ammonium thiocyanate on gold uptake by corn*

For this experiment, the set up was performed as described above. Agar growth medium was added with different compounds as follows: (a) 80 mg Au L<sup>-1</sup>; (b) 80 mg Au L<sup>-1</sup> + TU 1.00 x10<sup>-3</sup> M, and 80 mg L<sup>-1</sup> of gold + AT 1,00 x10<sup>-3</sup> M. TU and AT concentration was selected based on a screening experiment. For all experiments, three replicates were set up for statistical purposes.

### *Metal analysis*

After the growth period, the plantlets were washed with 0,01 M HNO<sub>3</sub>, rinsed with deionized water, and root and shoot elongation was recorded. Furthermore, plants were sectioned into roots and shoots. Plant tissues were oven dried at 70°C for 72 h, and dry weight was also recorded. Subsequently, samples were digested with 3 mL trace pure HNO<sub>3</sub> at 115° C using mi-

crowave assisted digestion (CEM Mars X, CEM Corporation, Mathews, NC). Later, the volume was adjusted to 15 mL and the gold content was determined using a Perkin Elmer Optima 4300 DV ICP/OES (Perkin Elmer Corporation, Shelton, CT) spectrometer using the wavelength of 277,595 nm.

### XAS studies

The biomass samples were frozen in liquid nitrogen and lyophilized using a Labconco Freeze-Dry System (Freezone 4.5, Labconco, Kansas City, MO). Data collection was performed on beamline 2-3 at the Stanford Synchrotron Radiation Laboratory (SSRL) using a 13 element Ge detector in fluorescence mode on the Au  $L_{III}$  ( $E_0$  11,918 keV), using an internal Au(0) metal foil for reference. Beamline operating conditions were as follows: an energy of 3,0 GeV, a current ranging from 80-100 mA, a silicon 220 double crystal monochromator (with a  $\phi$  90 orientation), and a 1,0 mm upstream slit. In addition, the beamline was detuned by 30% to reject higher order harmonics. The model compounds ( $KAuCl_4$  and AuOH) were diluted with boron nitride to obtain a homogenous mixture and a final gold concentration of 5%. The Au(0) model compound was a 1  $\mu$ m layer of Au(0) on Mylar tape. Ground plant tissues and diluted model compounds were packed into 1.0 mm aluminum sample plates previous to analysis.

### XAS Data Analysis

The WinXAS software and standard data reduction techniques were used to extract the gold XANES and EXAFS for the XAS spectra (Ressler, 1998). Spectra were first calibrated based on the energy of the ejected photoelectron from the internal Au(0) metal foil,  $E_0$  11,918 keV. The calibration was performed by taking a second degree derivative of the metal foil edge. Later, the background correction was done with a one degree polynomial fitting on the pre-edge region and a fourth degree polynomial to the post edge region. Subsequently, the samples were normalized to one absorption unit and XANES spectra were extracted from 11,85 to 12,05 keV. XANES spectra were fitted using LC-XANES with the model compounds Au(0) foil, AuOH, and  $KAuCl_4$ . The EXAFS were extracted from the background corrected normalized XAS spectra.  $E_0$  of the sample spectra were calculated from a second degree derivative of the sample edge to convert the spectra to k-space ( $\text{\AA}^{-1}$ ). A  $\mu$  fitting of the spectra was performed using a spline of 4 knots, and a k weight of 3 from 2,0 to 15,0  $\text{\AA}^{-1}$ . The EXAFS spectra were then Fourier transformed from 2,0 to 15,0  $\text{\AA}^{-1}$ . Sample spectra were then back transformed from 1,64 to 3,39

$\text{\AA}^{-1}$  to extract the first shell EXAFS. The back transformed EXAFS were then fitted using calculations from FEFF 8,00 to determine coordination numbers, interatomic distances,  $S_0^2$ , and energy shifts of the samples. Inputs for FEFF files were based on Au(0) because it was the highest component determined from the LC-XANES fittings. ATOMS software was used to create the crystal structure for bulk gold (Ravel, 2001).

## RESULTS AND DISCUSSION

### Effect of gold concentration on plant growth and gold uptake

The results showed that corn root elongation in control and treatments was around 15 cm (Figure 1). Gold treatments used in this investigation were not toxic to this plant part. However, shoot size was the same in controls and in kernels germinated in 80 mg  $L^{-1}$  (14 cm), while lower and higher Au levels provoked a decrease in shoot size (Figure 1). In addition, increasing gold concentration in the growth media yield to an increase in gold uptake by corn roots (Figure 2) and shoots (Figure 3). The highest uptake was observed in seedlings grown in 160 mg  $Au L^{-1}$ , where gold content was of 230 mg  $kg^{-1}$  (roots) and 6.8 mg  $kg^{-1}$  (shoots). Gold concentration in shoots of seedlings germinated and grown in 20 mg  $L^{-1}$  was below the detection limit (0,031 mg  $L^{-1}$ ). Given these results, 80 mg  $Au L^{-1}$  was chosen for further experiments.

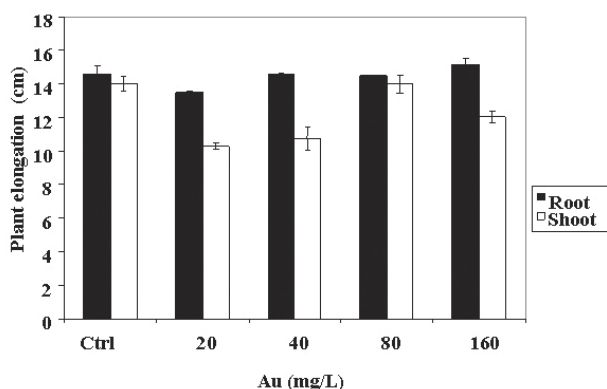


Figure 1. Root and shoot length of *Z. mays* (var. Golden) germinated and grown in 0-160 mg  $Au/L$  in agar media (error bars represent  $\pm$  Standard Error of the mean).

### Thiourea and ammonium thiocyanate promoted gold uptake in *Z. mays*

For this experiment, TU and AT were used as chemical amendments. Plant growth, (measured as plant elongation) was decreased by 50% in the presence of TU and by 30% in AT (data not shown). It was determi-

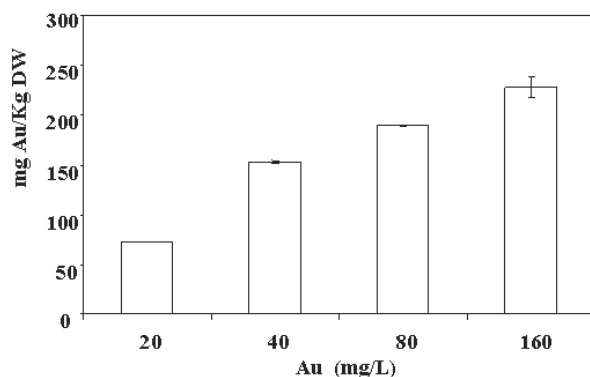


Figure 2. Gold concentration in roots of *Z. mays* (var. Golden) germinated and grown in 0-160 mg Au/L (error bars represent  $\pm$  S.E.).

ned that AT was more toxic for the var. Golden plants. Despite the toxicity of TU and AT for corn development, it was found that gold uptake was considerably enhanced with both compounds, especially in corn roots (Figure 4). Plants grown in gold alone took up 180 mg kg<sup>-1</sup> and 10 mg kg<sup>-1</sup> in roots and shoots, respectively. Addition of TU to the media, promoted gold uptake to levels of 1200 mg kg<sup>-1</sup> and 100 mg kg<sup>-1</sup> in the same order. In plants grown in gold and AT, the uptake was of 1500 mg kg<sup>-1</sup> (roots) and 5 mg kg<sup>-1</sup> (shoots). As these results show, more than 1 g Au kg<sup>-1</sup> dry roots was obtained. Sharma *et al.*, (2007) reported that roots of *Sesbania drummondii* were able to uptake from 1 to 9 g kg<sup>-1</sup>. (These researchers have proposed that the result could be due either to an accelerated gold uptake or to the synthesis of gold nanoparticles at root surface. Because TU assisted to uptake higher Au concentration in the aerial part and was less toxic to the plants, it can

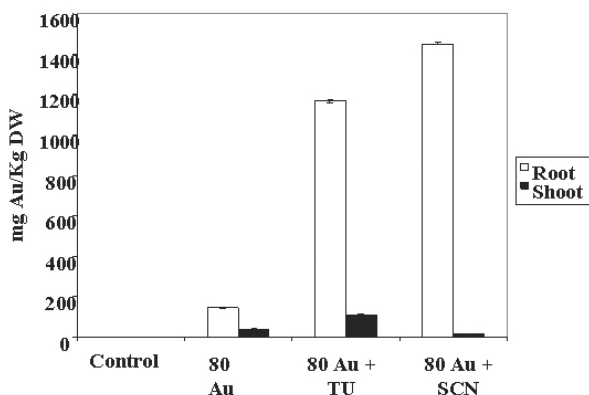


Figure 4. Gold concentration in roots and shoots of *Z. mays* (var. Golden) germinated and grown in 80 mg Au/L and TU or TSCN as chelating agent (error bars  $\pm$  represent S.E.).

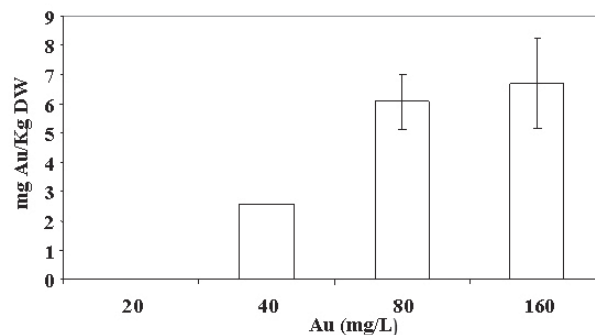


Figure 3. Gold concentration in shoots of *Z. mays* (var. Golden) germinated and grown in 0-160 mg Au/L (error bars represent  $\pm$  S.E.).

be concluded that this compound could be considered as a potential chemical amendment for gold phytomining by corn.

#### X-ray absorption spectroscopy revealed Au biotransformation in *Z. mays*

The XANES spectra for model compounds, Au(0), and roots of the Golden corn treated with 160 mg Au L<sup>-1</sup> are shown in Figure 5. The LC-XANES fittings showed that approximately 91% of the gold was present as gold(0) and about 9% was in a non-reduced form. Gold on/in the corn roots was reduced from Au(III) to Au(0). Similar results with other plants species and plant materials such as alfalfa plant, desert willow, oat biomass, wheat biomass, hops biomass, and alfalfa biomass reacted with Au(III) were reported in the literature (Gardea-Torresdey *et al.*, 2003; López *et al.*, 2005; Rodriguez *et al.*, 2007).

EXAFS spectra of the gold present in/on corn roots are shown in Figure 6. Results of the EXAFS fitting for bulk gold demonstrated that coordination in the first shell was of 12.0 neighboring gold atoms at approximately 2.86 Å (Table 1) which is a complete first shell coordination for face centered cubic metals. However, in corn roots the coordination number was approximately 9.5 neighboring gold atoms at approximately 2.86 Å, indicating an incomplete first coordination shell. The incomplete first coordination shell in EXAFS indicates that gold is present as a nano-phase. This has also been shown for gold reacted with living plants such as alfalfa and desert willow, and inactivated biological materials of alfalfa, oat, wheat, and hops (Gardea-Torresdey *et al.*, 2003; Lopez *et al.*, 2005; Rodriguez *et al.*, 2007). According to Borowoski equation (1997) the average size of 10.36 nm for gold nanoparticles were determined using a spherical model. Alfalfa

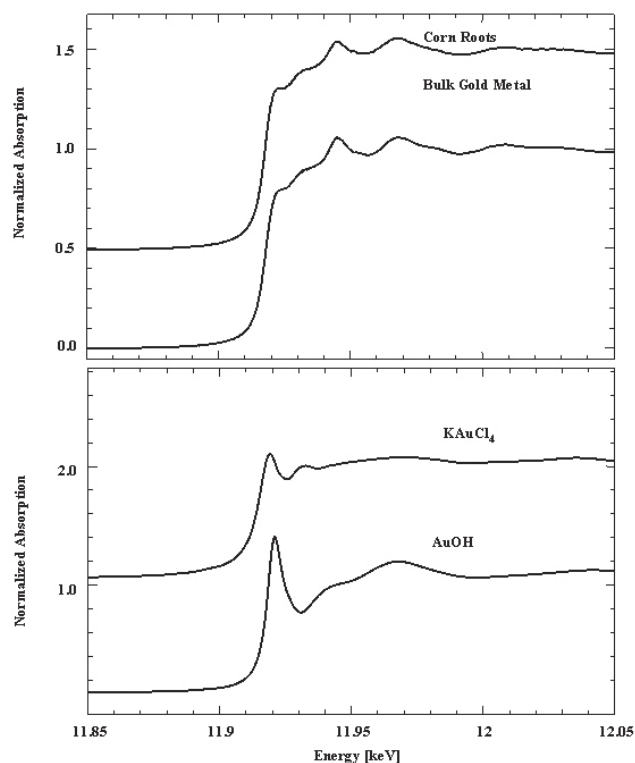


Figure 5. XANES spectra of gold model compounds and *Z. mays* (var. Golden) roots germinated and grown in 160 mg Au/L.

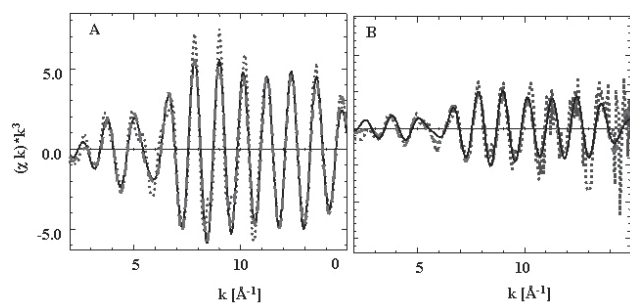


Figure 6. (A)  $L_{III}$  edge EXAFS of bulk gold metal model compound; (B)  $L_{III}$  edge EXAFS of gold on corn roots. Blue line represents the raw EXAFS, red line represents the back transformed first shell EXAFS and black line is the FEFF fitting of the first shell EXAFS.

and rattlebush produced gold nanoparticles between 6-20 nm, and 2-20 nm respectively (Gardea-Torresdey *et al.*, 2002; Sharma *et al.*, 2007); thus, average sizes were similar in these three plant species (corn, alfalfa, and rattlebush).

Table 1.  
FEFF fittings of gold found in bulk gold and Golden corn root samples.

Sample	Bond	CN	R(Å)	$\sigma^2(\text{Å}^2)$	$S_0^2$
Bulk Gold	Au-Au	12**	2.86	0.0078	0.87
Golden Corn Roots	Au-Au	9.5	2.86	0.0075	0.87**

\*\* indicated that the parameter was held constant for the fitting.

## CONCLUSIONS

Use of TU resulted in an increase of gold uptake by corn roots and shoots. XAS studies indicated that gold present on corn roots was indeed gold(0) nanoparticles. XANES studies showed that gold present on/in the roots was approximately 91% reduced gold, and 9% oxidized gold. In addition EXAFS from root samples showed that the nearest neighboring atom was a gold atom at an interatomic distance of 2.86 Å. Coordination number of 9.5 indicated the presence of gold nanoparticles, which size was calculated of 10.36 nm.

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## REFERENCES

- Anderson, C.W.N., Brooks, R.R., Stewart, R.B., Simcock, R., (1998). Harvesting a crop of gold in plants. *Nature* 395, 553-554.
- Anderson, C.W.N., Brooks, R.R., Chiarucci, A., LaCoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R., Stewart, R.B. (1999a). Phytomining for nickel, thallium and gold. *J. Geochem. Explor.* 67, 407-415.

- Anderson, C.W.N., Brooks, R.R., and Stewart, R.B. (1999b). Gold uptake by plants. *Gold Bull.* 32(2), 48-51.
- Anderson, C., Moreno, F., Meech, J. (2005). A field demonstration of gold phytoextraction technology. *Miner. Eng.*, 18(4), 385-392.
- Borowski, M. (1997). Size determination of small Cu-clusters by EXAFS. *J. Phys. IV*, 7 (1-2), C2.259-C2.260.
- Brooks, R. R., Chambers, M. F., Nicks, L. J., Robinson, B. H. (1998). Phytomining. *Trends Plant Sci.* 3(9), 359-362.
- Deng, T. L., Liao, M. X., Wang, M. H., Chen, Y. W., Belzile, N. (2001). Technical note enhancement of gold extraction from biooxidation residues using an acidic sodium sulphite-thiourea system. *Miner. Eng.* 14(2), 263-268.
- Gardea-Torresdey, J.L., Parson J. G., Gomez, E., Peralta-Videa, J., Troiani, H. E., Santiago, P., Yacaman, M. J. (2002). Formation and growth of Au nanoparticles inside live alfalfa plants. *Nano Letters* 2(4), 397-401.
- Gardea-Torresdey, J.L., Gómez, E., Peralta-Videa, J.R., Parsons, J.G., Troiani, H., Jose-Yacaman, M. (2003). Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir*, 19(4), 1357-1361.
- Hiskey JB. (1984) Thiourea leaching of gold and silver-technology update and additional applications. *Minerals Metall. Process.* 11, 172-182.
- Khandelwal, S. K., Gupta, N. K., Sahu, M. P. (2002). Effect of plant growth regulator on growth, yield and essential oil production of henna (*Lawsonia inermis* L.). *J. Horti. Sci. Biotechnol.* 77(1), 67-72.
- Lamb, A. E., Anderson, C. W., Haverkamp, R. G. (2001). The Induced Accumulation of Gold in the Plants *Brassica Juncea*, *Berkheya Coddii* and *Chicory*. *Chem. N. Z.* 65(2), 34-36.
- López, M.L., Gardea-Torresdey, J., Peralta-Videa, J.R., De la Rosa, G., Armendariz, V., Herrera, I., Troiani, H., Henning, J. (2005). Gold Binding by Native and Chemically Modified Hops Biomasses. *Bioinorg. Chem. Appl.* 3(1-2), 29-41.
- Msuya, F.A., Brooks, R.R., Anderson, C.W.N., (2000). Chemically-induced uptake of gold by root crops: its significance for phytomining. *Gold Bull.* 33(4), 134-137.
- Peralta, J.R., Gardea-Torresdey, J.L., Tiemann, K.J., Gomez, E., Arteaga, S., Rascon, E., Parson, J.G. (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bull. Environ. Contam. Toxicol.* 66, 727-734.
- Philip, D. (2009). Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. *Spectrochim. Acta Part A*, 73(2), 374-381.
- Ravel B. (2001) ATOMS: crystallography for the X-ray absorption spectroscopist. *J. Synchrotron Radiat.* 8(Pt 2), 314-6.
- Ressler, T. (1998) WinXAS: a program for x-ray absorption spectroscopy data analysis under MS-Windows. *J. Synchrotron Radiat.*, 5(2), 118-122.
- Rodríguez, E., Parsons, J.G., Peralta-Videa, J.R., Cruz-Jiménez, G., Romero-González, J., Sánchez-Salcido, B.E., Saupé, G.B., Duarte-Gardea, M., Gardea-Torresdey, J.L. (2007). Potential of *Chilopsis linearis* for gold phytomining: Using XAS to determine gold reduction and nanoparticle formation within plant tissues. *Int. J. Phytorem.*, 9-2, 133-147.
- Rodríguez, E., Peralta-Videa, J.R., Sánchez-Salcido, B., Parsons, J.G., Romero, J., Gardea-Torresdey, J.L. (2007) Improving gold phytoextraction in desert willow (*Chilopsis linearis*) using thiourea: a spectroscopic investigation. *Environ. Chem.* 4(2) 98-108
- Sahu, M. P., Solanki, N. S., Dashora, L. N., (1993). Effects of thiourea, thiamine and ascorbic acid growth and yield of maize (*Zea mays* L.). *J. Agron. Crop. Sci.* 171(1), 65-69.
- Sharma, N.C., Sahi, S.V., Nath, S., Parsons, J.G., Gardea-Torresdey, J.L., Pal, T. (2007). Synthesis of plant-mediated gold nanoparticles and catalytic role of bio-matrix-embedded nanomaterials. *Environ. Sci. Technol.*, 41(14), 5137-5142.
- USGS, 2008. United States Geological Survey web page: <http://toxics.usgs.gov/definitions/biotransformation.html>