INNOVATIVE TECHNOLOGY TO RECOVER GOLD(III) FROM AQUEOUS SOLUTIONS BY USING MEDICAGO SATIVA(ALFALFA)

JL Gardea-Torresdey[a], K.J. Tiemann[a], G. Gamez[a], K. Dokken[a], M.J. Yacaman[b]

Department of Chemistry and Environmental Sciences and Engineering
[a]University of Texas at El Paso, El Paso, Texas 79968; Phone: (915) 747-5359, Fax: (915) 747-5748; [b]Instituto Nacional de Investigaciones Nucleares, KM. 36.5 Carretera Federal Mexico-Toluca, Municipio de Ocoyoacac Estado De Mexico - CP 52045

ABSTRACT

There is a need for the development of a system that can extract gold out of aqueous solutions without the use of hazardous chemicals. A biorecovery system using alfalfa biomass may be the answer for the recovery of gold(III) from aqueous solutions in an environmentally friendly manner. Batch laboratory experiments indicate that alfalfa binds gold(III) quickly and in a pH-independent manner. Gold(III) binding capacity experiments performed with the alfalfa roots and shoots have shown the following results: 40.9 mg gold per gram of shoot tissues; 18.7 mg gold per gram of root tissues. We were able to recover up to 99.1% of the bound gold metal by treatment with 0.2 M acidic thiourea. Surprisingly we discovered that the bound gold turned purple in color, meaning that gold(III) had been reduced to gold(0). This was confirmed by transmission electron microscopic analysis (TEM). In addition, column experiments were performed with silica-immobilized alfalfa to determine the gold(III) binding ability under flow conditions. This in turn could constitute an innovative pollution prevention technology to reclaim gold from natural and industrial processes and prevent the use of hazardous chemicals that might contaminate groundwaters.

Key words: *pollution prevention, heavy metal binding, gold, phytofiltration, alfalfa*

INTRODUCTION

The allure of gold and other precious metals to the mining industry has increased within the last decade primarily as a result of their high metal prices (Lucas, 1985). As mining technology has increased, many old and abandoned mines have been reopened. Technologies such as heap leaching have established themselves as economical methods for the recovery of precious metals from low grade mineral deposits. Amalgamation, cyanidation, and thiourea leaching are some of the methodologies that are used to separate gold from their ores (Addison, 1980; Hiskey, 1985; Cho et al., 1983; Hisshion et al., 1984; Deschenes et al., 1989, Zipperian et al., 1989). These gold recovery processes require the use of hazardous chemicals, such as cyanide, which pose a serious threat to the public's health (White, 1985). Due to these health concerns, there is a need for the development of an environmentally friendly alternative for the recovery of precious elements.

It has been known for quite some time that plants have the unique ability to uptake gold from soils and accumulate gold in their tissues. As early as 1900, gold was detected in plant tissues as a method of fire ashing was used to obtain gold beads from hardwood trees (Girling et al., 1980). It has also been suggested that black gold deposits might be due to the dissolution of fine gold particles in water by humic substances derived from decaying vegetable matter (Rapson, 1982). Dissanayake and coworkers found gold and platinum accumulated in natural deposits of peat and algal biomats (Dissanayake et al., 1984). The peat was found to contain gold in the colloidal phase

and platinum was bound on the humic material. In addition, Gardea-Torresdey and coworkers used modified biomass to construct a biosensor for the detection of gold in solution (Gardea-Torresdey et al., 1988). Therefore gold mineralization by biomaterials from aqueous solutions may be an alternative to the use of hazardous chemicals.

Medicago sativa (alfalfa) has been found to tolerate heavy metals contaminated soils (El-Kherbawy et al., 1989; Cajuste et al., 1991; Baligar et al., 1993; Rechcigl et al., 1988). Gardea-Torresdey et al., have shown that alfalfa is a potential source for removal and recovery of heavy metal ions (Gardea-Torresdey et al., 1996a; 1996b; 1996c). Batch laboratory experiments have determined that alfalfa biomass possesses the ability to bind various heavy metal ions from aqueous solutions. In addition, considerable amounts of the bound metal ions were recovered from the reusable silica-immobilized biomaterial. This biorecovery process for heavy metals could also be utilized for the removal and recovery of gold(III) from aqueous solution. Therefore, the development of a new recovery method using alfalfa biomass to collect gold(III) from industrial waste sources might not only reduce the risk of hazardous chemical use, but it may also be a more cost-effective method as well.

The objective of this study was to investigate the ability of alfalfa roots and shoots to bind gold(III) (as tetrachloroaurate(III)). Batch laboratory pH profile, and time dependency and capacity experiments were performed to determine the binding characteristics of the Malone shoots and roots to gold(III). Batch recovery experiments were carried out to determine the amount of gold that could be recovered after being bound by the alfalfa shoots and roots. In addition, column experiments were performed with silica-immobilized Malone alfalfa shoots to determine the extraction and recovery ability of gold(III) under flow conditions. These studies may be useful in the development of an innovative method for gold removal and recovery from mining leachate, electroplating, and smelting waste waters through phytofiltration.

MATERIALS AND METHODS

Alfalfa Collection

The Malone population of alfalfa biomass was selected from previous studies for its abundance and metal binding abilities. The plant tissues were collected from controlled agricultural fields at New Mexico State University near Las Cruces, New Mexico. The plants were removed from the soils, washed throughly to remove any debris and the roots were separated from the shoots (stems and leaves). The samples were then oven dried at 90°C for one week. The dried samples were ground to pass through a 100-mesh screen using a Wiley mill.

pH Profile for Gold(III)Binding

Batch laboratory techniques were used for the pH studies. A 250-mg sample of biomass was washed twice with 0.01 M hydrochloric acid (HCl) to remove any debris or soluble

biomolecules that might interact with gold ions. Each biomass sample was resuspended in 50 mL of 0.01 M HCl with tissue concentration of approximately 5 mg per mL. The pH was adjusted to 1.0, allowed to equilibrate, and 2 mL aliquots of the suspension were transferred into three 5 mL plastic tubes. The pH was then adjusted (with NaOH) and allowed to equilibrate at pH 2.0, 3.0, 4.0, 5.0, and 6.0, and 2 mL aliquots of the suspension at each pH were transferred into 3 new tubes for each pH. The suspensions were centrifuged at 2,500 rpm for 5 minutes and the supernatants were examined to determine if soluble materials in solution could be responsible for gold binding. A gold(III) solution of 0.1 mM (made from potasium tetrachloroaurate(III) ($KAuCl_4$)) was prepared and the pH adjusted to 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0. At each pH, 2 mL of the metal solution were added to the respective pH biomass pellet, and to the separated supernatant solutions. In addition, at each respective pH, 2 mL of the 0.1 mM metal solution were transferred to 3 tubes for controls. All the tubes were equilibrated on a rocker for 1 hour. The samples were then centrifuged at 3,000 rpm for 5 minutes and the supernatants for the pellets were transferred to clean tubes. Final pHs for all tubes were recorded and analysis for gold was performed by flame atomic absorption spectroscopy.

Time Dependence Studies for Gold(III) Binding

A 500-mg sample of biomass was washed twice with 0.01 M HCl to remove any debris or soluble biomolecules that might interact with metal ions. Each biomass sample was resuspended in 100 mL of deionized water with tissue concentrations of approximately 5 mg per mL. The solution was then adjusted to pH 2.0 and allowed to equilibrate. Two mL aliqouts of the suspension were transferred to 24 tubes; 3 tubes for each time interval of 5, 10, 15, 20,30, 45, 60, and 90 minutes. After centrifugation and decantation, 2 mL of 0.3 mM gold(III) solution was added to each of the tubes and controls. All the tubes were equilibrated by rocking and were removed at the appropriate time intervals. The samples were then centrifuged at 3,000 rpm for 5 minutes and the supernatants from the pellets were transferred to clean, respective tubes. Final pHs for all tubes were recorded and gold analysis was performed by flame atomic absorption spectroscopy.

Adsorption Studies for Gold(III) Binding

Samples of 100 mg of biomass were washed twice with 0.01 M HCl and washings were collected and weighed to determine biomass loss. Washed biomass was resuspended in 20 mL of deionized water and the pH adjusted to 2.0 (tissue concentration of approximately 5 mg per mL). Two mL of the suspension were transferred to 3 tubes and then centrifuged. Two mL aliqouts of 0.3 mM gold(III) solution (at pH 2.0) were added to each of the tubes and controls. After equilibration for 10 minutes, the tubes and controls were centrifuged, and the decanted supernatants were stored for gold analysis and again 2 mL of 0.3 mM gold(III) solution were added until the biomass became saturated.

Desorption of Bound Gold

In order to remove the bound gold from the alfalfa biomass, pellets from binding capacity studies with the adsorbed gold were exposed to 2 mL of 0.2M thiourea (in 0.2 M HCl), equilibrated by rocking for 10 minutes, and then centrifuged as indicated by Gardea-Torresdey et al., (Gardea-Torresdey et al., 1996c). The resulting supernatant was collected for analysis and diluted as required to stay within the calibration range. All gold analysis was performed by flame atomic absorption spectroscopy.

Immobilized Alfalfa Biomass and Column Experiments

The immobilization of the Malone alfalfa biomass was performed as indicated previously by Gardea-Torresdey et al. (Gardea-Torresdey et al., 1996b;1996c). Samples of 5 g were washed twice with water and the cell debris were removed by centrifugation. The following part of this experiment is similar to that reported before for the binding of copper and nickel to different species of *Medicago sativa* (Gardea-Torresdey et al., 1996b;1996c). One bed volume of solution that is passed through the column is equivalent to the volume of immobilized biomass within the column. In this case, the volume of immobilized biomass used was 6 mL; therefore, one bed volume is equal to 6 mL. The 0.1mM gold(III) solution at pH of 2.0 was passed at a flow rate of 2 mL per minute.

Multi-metal Column Experiments

Column experiments were performed as described above using a multi-metal solution containing 0.1 mM of the each of the following metal ions: cadmium(II), copper(II), chromium(III), gold(III), lead(II), nickel(II), and zinc(II). The metal solution was prepared from the corresponding salts: Cd(NO3)2,CuSO4, Cr(NO3)3, KAuCl4, Pb(NO3)2, Ni(NO3)2, and ZnCl2. The 0.1mM multi-metal solution at pH of 2.0 was passed at a flow rate of 2 mL per minute through the column. This experiment was carried out 3 times for quality control. In addition, the experiment was repeated with the 0.1mM multi-metal solution at pH 5.0. Analyses for metal ions was performed by flame atomic absorption spectroscopy.

Recovery of Gold from Column Experiments

To remove the bound metal from the immobilized Malone alfalfa shoots, 10 bed volumes of 0.2M thiourea (in 0.2 M HCl) were passed through the column at a flow rate of 2 mL per minute. Each effluent bed volume was collected and analyzed by flame atomic absorption spectroscopy. The amount of metal recovered in each bed volume of effluent was summed and the total was taken to be the total amount of gold recovered from the column.

Transmition Electron Micrographs

The Transmition Electron Micrographs (TEM) were obtained at the Instituto Nacional de Investigaciones Nucleares in Toluca, Mexico. The images were obtained using a TEMJEOL 100cx instrument with a resolution of approximately 5A.

Metal Analyses

The gold content in all the experiments was performed by using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction using a wavelength of 242.8 nm. The following wavelengths were used for the other metal ions studied from the mixed metal solutions: cadmium, 228.8 nm; copper, 327.2 nm; chromium, 358.2 nm; nickel, 352.5: lead, 283.3 nm; and zinc, 213.9 nm. The instrument response was periodically checked with known standards. A calibration curve was obtained with a correlation coefficient of 0.98 or greater. The samples were read three times and the mean value and relative standard deviation were computed. Samples were diluted as required to remain within the calibration linear range. An impact bead was utilized to improve the sensitivity. The difference between the initial gold concentration and the remaining gold concentration was assumed to be bound to the biomass.

RESULTS AND DISCUSSION

Figure 1 shows that gold(III) binds to the alfalfa biomass in an almost pH-independent manner. Figure 2 shows the time required to bind gold(III) by the Malone alfalfa shoots and roots. As seen in Figure 1, the graph shows that Malone alfalfa biomass binds gold(III) strongly throughout the pH range. This lack of pH dependence (pH 2.0 to 6.0) suggests that the gold is binding to the alfalfa in a covalent manner, but rather electrostatic interactions. (Gardea-Torresdey et al., 1996a; 1996b; 1996c; 1996d). This trend in binding is characteristic of the soft classification of gold(III) ions. This feature might be useful for the separation of gold from other heavy metal ions by using alfalfa biomass since other metals do not bind to alfalfa in a covalent manner rather than through electrostatic interactions as seen for other metals (Gardea-Torresdey et al., 1996a; 1996b; 1996c; 1996d). Because the binding of gold by alfalfa biomass is characteristic of gold(III) ions when they bind to soft ligands, alfalfa may be used to recover gold from solutions at low pH (Green et al., 1986). Also, alfalfa shoots seem to bind more gold(III) than the roots. Figure 2 shows the binding of gold(III) by the biomass is rapid (within five minutes) and remains fairly stable for 90 minutes. However, the alfalfa shoots appear to start binding more gold after 45 minutes. Since all of the soluble components were eliminated in prior washing, the binding must be due to the alfalfa biomass. Because the alfalfa plant tissues were inactivated during drying, the rapid binding of the gold ions may be due to functional groups located on the plant cell wall and not due to active cellular processes. In addition, it was observed that a purple color appeared on the biomass after a period of one-hour reaction with the 0.3 mM gold(III). Previous studies have determined that the development of a purple color by biomaterials under similar conditions is due to the reduction of Au(III) to Au(0), which is similar to the "Purple of Cassius" in ancient times (Lujan et al., 1994; Chow et al., 1994; Druff et al., 1987). If the bound gold(III) is then being reduced to gold(0), causing the gold to bio-plate out of solution, then the once occupied binding site might be freed and additional gold may bind. This may explain the increase in binding seen after the one-hour interval. Transmission

electron microscopic analysis (TEM) was performed on the purple-colored particles. Figure 3 is one micrograph depicting the decahedral-shaped colloidal gold particles found after the formation of the purple color. Figure 4 is the second micrograph depicting the pseudo-trigonal-shaped particles found after the formation of the purple color. These decahedral and pseudo-trigonal particles are characteristic of colloidal gold (Chow et al., 1994; Druff et al., 1987). These findings support the hypothesis of Au(III) reduction by the alfalfa biomass to Au(0). Similar observations have been found with gold binding by other biomaterials (Lujan et al., 1994).

Binding capacity experiments were performed to determine the amount of gold that could be removed from aqueous solutions. Table 1 shows the amount of gold bound by the Malone alfalfa shoots and roots. Saturation of the biomass was achieved after 8 gold binding cycles. Table 1 shows that the Malone shoots have a higher binding capacity (40.1mg/g) than the roots (18.7mg/g). Because the pH profiles did not indicate a trend in pH-dependent binding, the bound gold was recovered using 2 mL of a 0.2 M thiourea combined with 0.2 M HCl solution. As seen in Table 1, the recovery of bound gold was excellent, ranging from 87.6 % for Malone alfalfa shoots to 99.1 % for Malone alfalfa roots.

In addition, column experiments were performed with silica-immobilized alfalfa to determine the gold(III) binding ability under flow conditions. After passing 200 bed volumes of a solution of 0.3mM gold(III) at pH 2.0 (approximately 60 ppm) through three different packed columns of silica-immobilized alfalfa, the biomass was still binding. The columns of silica-immobilized alfalfa were able to bind an average of 9,272 parts per million (ppm) of gold from solution. After exposing the columns to 6 bed volumes of 0.2 M thiourea (in 0.2M HCl), we were able to recover an average of 73% of the bound gold. In order to determine if gold binding is affected by the presence of other metal ions, multi-metal column experiments were performed. Figure 5 shows the average amount of metal bound on three columns after 120 bed volumes had been passed at pH 2.0. As can be observed from Figure 5, nearly 2250 ppm of gold(III) were bound by the column containing immobilized alfalfa, where only 240 ppm of Pb(II), 100 ppm Zn(II), and nearly 30 ppm of Cd(II), Cr(III), Cu(II), Ni(II) were bound. Therefore, the silica-immobilized alfalfa has the ability to selectively bind gold(III) in acidic conditions. Figure 6 shows the average amount of metal bound on three columns after 120 bed volumes had been passed at pH 5.0. From Figure 6 it can be seen that only 730 ppm of gold(III) was bound by the column containing immobilized alfalfa, where the binding of the following metals was 850 ppm of Pb(II), 350 ppm Cu(II), 250 ppm of Cd(II), 160 ppm Cr(III), 75 ppm Zn(II), and 30 ppm Ni(II). Consequently, by passing a multi-metal solution through a series of columns at low pH, gold(III) could be selectively removed from the other metal ions through a passive process. This in turn could constitute an innovative pollution prevention technology to reclaim gold(III) from natural and industrial wastewaters and prevent the use of hazardous chemicals that might contaminate groundwaters.

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Table 1. Gold binding capacity for inactivated Malone shoot and root biomass.

Biomass	Capacity (mg gold / g Biomass)	% Gold Recovered
Shoots	40.1 mg/g	87.6%
Roots	18.7 mg/g	99.1%

NOTE: These experiments were performed with a 0.3mM gold (III) solution at pH 2.0.

Figure 1. Effect of pH on the binding of gold (III) by Malone alfalfa shoots (■) and roots (Δ). The biomass was shaken for one hour with 0.1mM tetrachloroaurate(III)

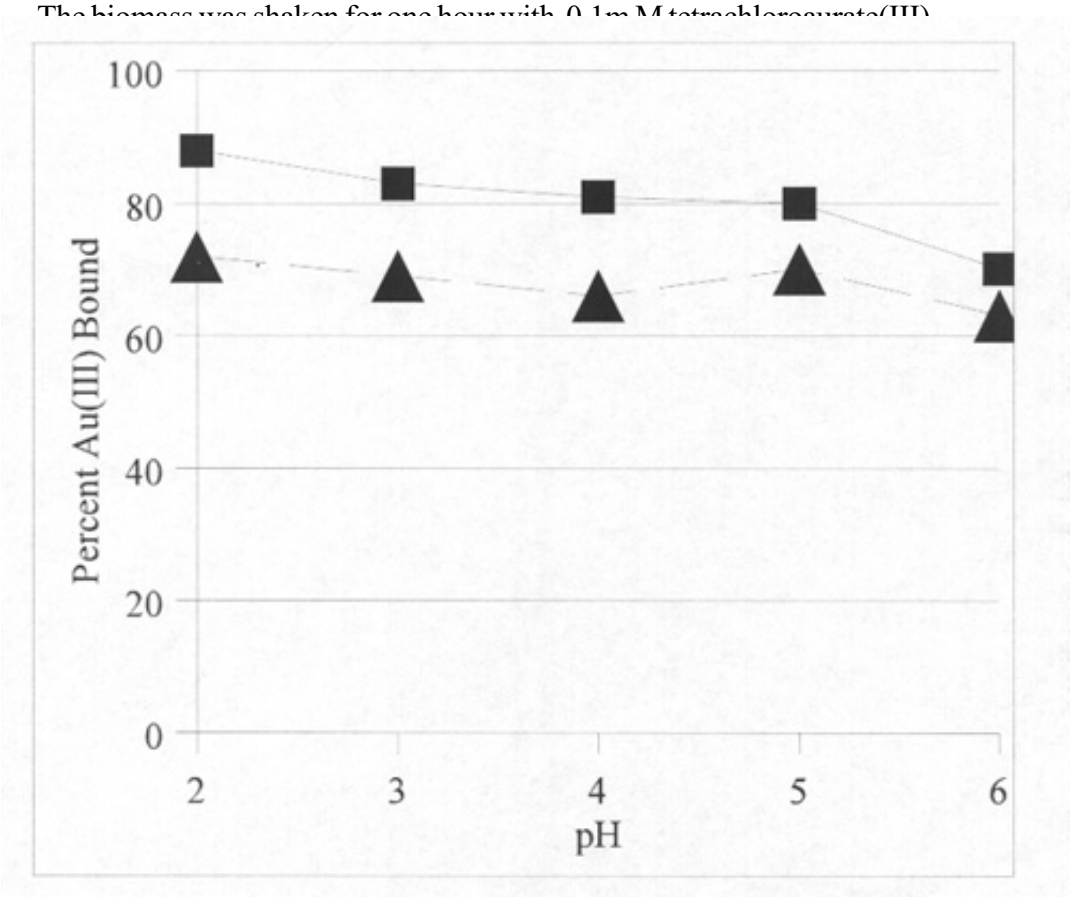

Figure 2. Time Dependence for gold (III) binding by Malone alfalfa shoots (■) and roots (Δ). The biomass was shaken for each time interval with 0.3 mM tetrachloroaurate(III) at pH 2.0.

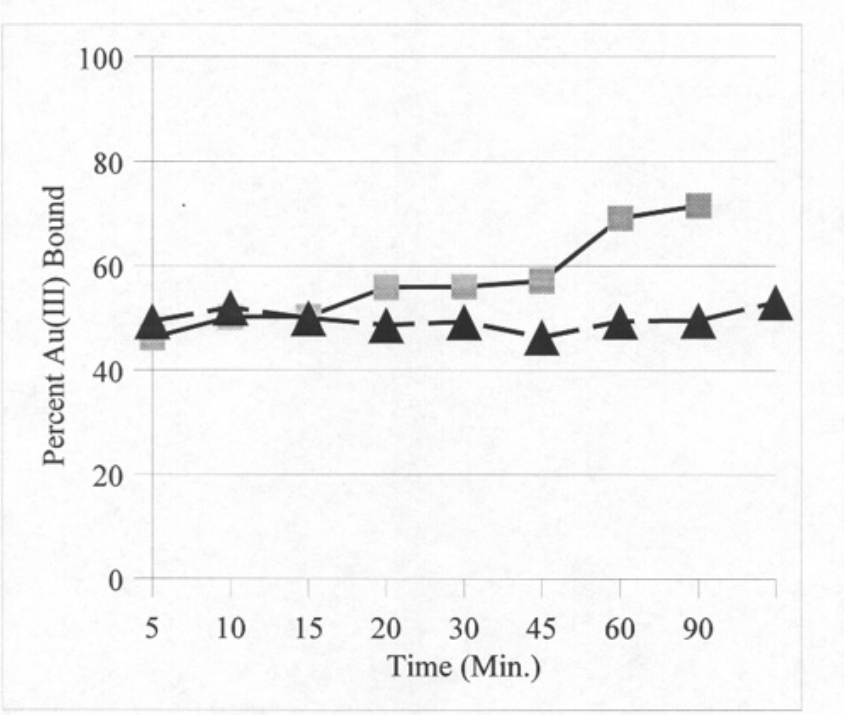

Figure 3. Decahedral colloidal particles formed when tetrachloroaurate(III) was exposed to Malone Alfalfa shoot biomass for over one hour.

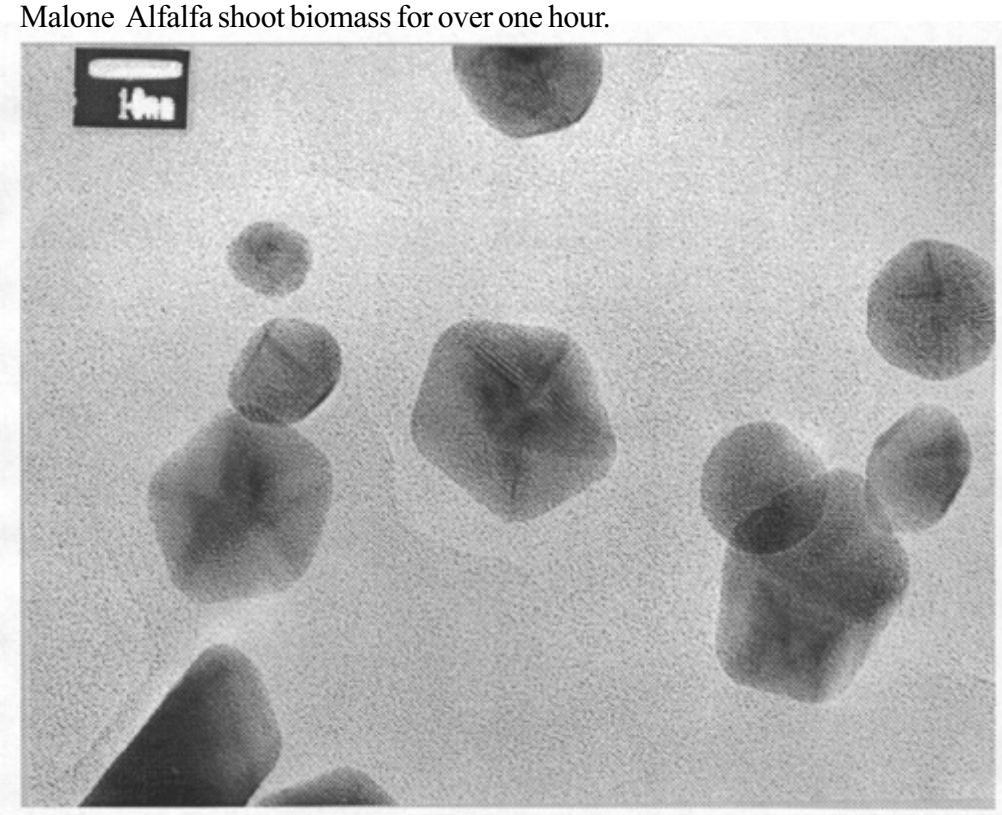

Figure 4. Pseudo-Trigonal colloidal particles formed when Tetrachloroaurate(III) was exposed to Malone shoot biomass for over one hour.

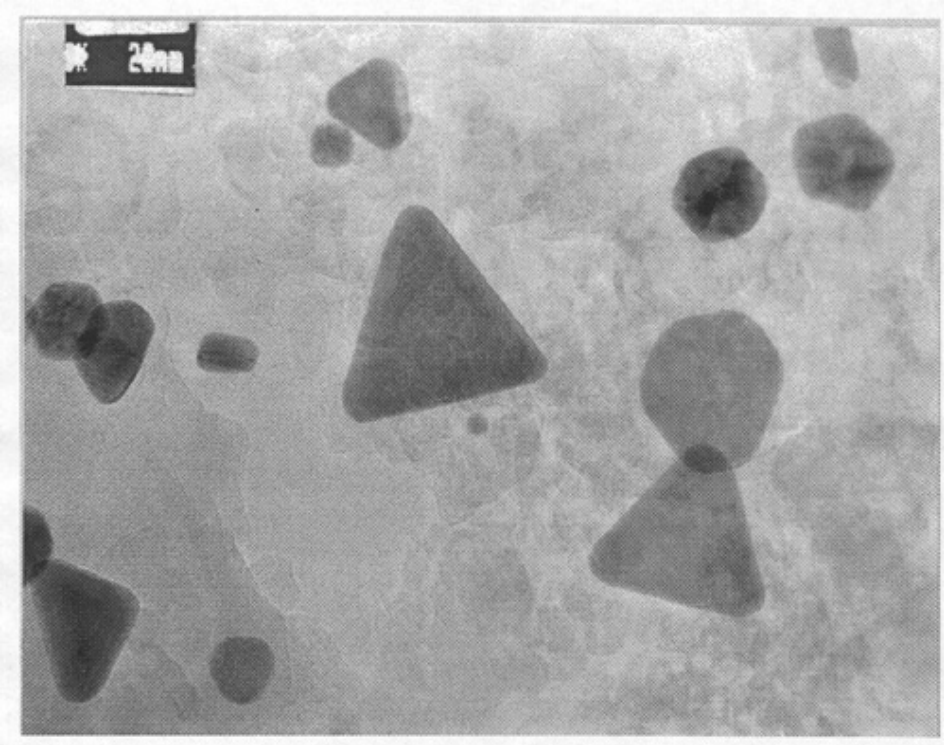

Figure 5. Effects of the presence of multi-metals on gold (III) binding by silica-immobilized Malone alfalfa shoots The columns were exposed to 120 bed-volumes of 0.1mM multi-metal solution at pH 2.0.

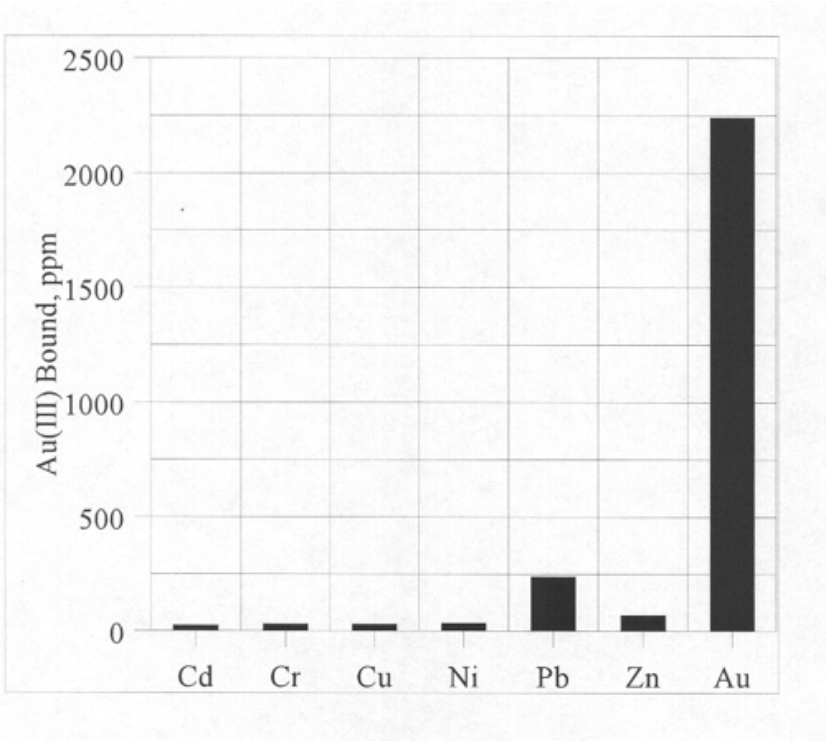

Figure 6. Effects of the presence of multi-metals on the binding by silica-immobilized Malone alfalfa shoots The columns were exposed to 120 bed-volumes of 0.1mM multi-metal solution at pH 5.0.