GOLD, SILVER, AND COPPER PHYTOEXTRACTION BY *HELIANTHUS ANNUUS* L. (SUNFLOWER) GROWN ON MINE TAILINGS AMENDED WITH PLANT GROWTH REGULATORS AND CYANIDE

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Keywords: Rooting, phytoextraction, mine tailings, *Helianthus annuus* L., gold, copper, silver

ABSTRACT

Phytoextraction has been suggested as aviable technique for gold recovery from some areas of mine tailings and mineralized soils. Plant Growth Regulators have been used due to their potential to improve rates of metal phytoextraction. However, no studies have specifically described the efficacy of exogenous plant growth regulators on the process of gold phytoextraction for plants grown in mine tailings. To evaluate the influence of rooting compounds (exogenous plant growth regulators) on Au, Ag, and Cu phytoextraction, two commercial products containing a mix of NaphtaleneAcetic Acid (NAA) and Indole-3-Butyric Acid (IBA) as active agents (auxins) were tested in a shade-house. *Helianthus annuus* (sunflower) were grown on mine tailings with a gold concentration of 2.30 mg/kg and high contents of Cu and Fe, over 7 weeks. Solutions prepared with two commercial PGR compounds were applied to the pots two weeks after seeding. Our results show that PGR treatment can increase the average Cu concentration up to 313 %, the average Ag concentrations up to 150 %, and the average Au concentrations up to 36 % in *H. annuus* relative to no treatment controls after the application of NaCN to the pot to induce metal solubility and uptake. This is the first evidence of the potential benefit of using rooting compounds (exogenous plant growth regulators) to improve the Au, Ag and Cu concentration in plants grown at mine tailings. The application of our results could directly impact on the profitability of the phytomining technique where they were verified in the field.

INTRODUCTION

In 1998, it was reported that plants of *Brassica juncea* (L) Czern. could be forced to accumulate Au to concentrations as high as 57 mg/kg of dry matter after suitable treatment of the substrate in which they were growing[1]. This technique is known as induced hyperaccumulation[2; 3] and supported the development of a novel mining technique named gold phytomining. Phytomining is the use of living plants that concentrate a metal in tissues and has been proposed as an alternative for the extraction of metals such as Au, Ni and Tl[4]. The possibility of converting phytomining in a profitable activity has been investigated by several research groups worldwide during the last years[5; 6; 7; 8; 9]. The profitability of this technique depends of factors such as the dry matter yield of the crop and the metal concentration accumulated in plant tissues, as well as gold prices in the market and agronomic and industrial production costs[10].

The application of exogenous plant growth regulators was examined in a recent study as a technique to increase the efficiency of plant metal extraction from contaminated soils[11]. Reported results showed plants such as *Alyssum murale* are sensitive to cytokinins treatment and that this treatment is potentially useful in increasing overall phytoextraction capability by increasing biomass. Other works have investigated the use of phytohormones or Plant Growth Regulators (PGR) to enhance the phytoextraction of metals such as Au[7] and Ni[12]. Phytohormones are endogenous bioactive substances in plants which control various processes of plant development[13] and are divided in gibberellins, cytokines, and auxins. In 2013, a study was conducted to evaluate the effect of two types of phytohormones (gibberellins and cytokines) on the Ni phytoextraction capacity of four *Alyssum* species[12]. Results showed that phytohormones application to plants has good potential to increase the efficiency of metal recovery from soil by phytoextraction.

It is known that a good root system is necessary for plants to absorption both nutrients as well as other metals that are not essential for life but are contained within the soil media. Auxins are substances that generate a plant response to influence the development of the root system. Phytohormones have an important role in plant development[14] and were initially identified as the bioactive substance that induces roots in plant tissue culture[15]. Thus, specific phytohormones generate specific responses in plants. It is possible that auxins can also serve as an important factor in improving of the levels of Au absorption by plant species grown on substrates generated by mining. Following on from this possibility, in 2013, Kulkarni and colleagues reported the use of auxins, gibberellins and cytokines to improve the Au uptake by *B. juncea* grown in a hydroponic solution. Cabello-Conejo and colleagues reported in 2013 the use of gibberellins and cytokines to induce improvement in Ni phytoextraction.

Phytomining is a technique that could be made more efficient through improvement of the phytoextraction processes for metals such as Au, Ni, Tl, and Ag. This technique has been proposed for application to both mine tailings as mineralized soils[1; 10]. However, no study has reported the effect of auxins applied when plants are grown on Au, Ag, and Cu mine tailings. Although the mine tailings contain heavy metal such as Cu and Hg that can be toxic for plants in certain concentrations, it is possible that improved rooting could improve the phytoextraction processes when plants are grown in these substrates. Based on this hypothesis, the study described in this paper was conducted to evaluate the influence of a varying concentration of each of two auxins plant growth regulator treatments to improve Au, Ag, and Cu uptake by *H. annuus* (sunflower) plants grown on mine tailings amended with NaCN to induce the uptake of gold.

MATERIALS AND METHODS

In order to evaluate the influence of two rooting products which contain auxins on Au uptake, *H. annuus* (sunflower) plants were grown on mine tailings from the San Jose de Gracia mine located in the Northwest portion of Mexico. One of three rates of each of two auxins plant growth regulator treatment was applied to these pots, and all pots were treated with NaCN at the end of the experiment to induce uptake of metals. A control treatment received NaCN but not PGR treatment. The physical-chemical characteristics of this substrate were determined in the laboratory (**Table 1**).

Table 1. Physical-chemical characterization of the substrate from the San Jose de Gracia tailings dam used for the PGR experiment

Parameter	Value	Unit
pH	7.8	------
EC	1077	mS/cm
Bulk density	1.3	t/m^3
OM	1.6	%
Au	2.30	$mg \cdot kg^{-1}$
Ag	49.83	$mg \cdot kg^{-1}$
Cu	163.11	$mg \cdot kg^{-1}$
Inorganic N	1.82	$mg \cdot kg^{-1}$
K	217.33	$mg \cdot kg^{-1}$
Ca	1809.8	$mg \cdot kg^{-1}$
Mg	1493.91	$mg \cdot kg^{-1}$
Na	49.77	$mg \cdot kg^{-1}$
Fe	10581.75	$mg \cdot kg^{-1}$
Mn	304.23	$mg \cdot kg^{-1}$
Zn	41.52	$mg \cdot kg^{-1}$

The plant available concentrations of the base cations K, Ca, Mg and Na were quantified through extraction with neutral 1M ammonium acetate and subsequent analysis of the extract solution by flame atomic absorption spectrometry. Total N and total P in the tailings were quantified using the Kjendahl-salicylic acid and HCl-NH_4F extraction methods respectively[16]. The organic matter content was estimated by loss on ignition at 550° C overnight in a muffle furnace. Tailings pH was measured in water at a soil to water ratio of 10:25 using a pH METER (model PHM83 AUTOCAL) after overnight equilibration.

A density of 10 sunflower plants/m^2 [17] was grown in a total of 33 pots, each containing 3.5 kg of homogenized tailings substrate. Plants were grown during 7 weeks in a shade-house with a temperature range of 24-38° C and environment humidity of 40-60 %. Two weeks after seeding, two rooting compounds (commercially known as Pro-root® and Alga-root®) each containing auxins, especially NAA, were applied to replicate pots to increase root mass and theoretically, the potential for metal uptake. The content of the two products is shown in **Table 2**.

Table 2. The content of two rooting products containing auxins that were applied to mine tailings supporting the growth of *H. annuus* plants. The content is as reported by the manufacturer.

	Product	
Content reported	**Pro-root**	**Alga-root**
NaphtaleneAcetic acid	2800 ppm	2000 ppm
Indole Butyric Acid	200 ppm	1000 ppm
Indole Acetic Acid	NR	400 ppm
Fulvic Acids	2 %	1 %
Usable Phosphorous	55 %	4 %
Total Nitrogen	11 %	NR

NR= Data no reported.

To prepare the Pro-root treatment, a total of 0.87 g of content of the product was diluted in 350 ml of water and then, five plant containers (replicate pots) were treated with 10 ml, five plant containers were treated with 20 ml and five plant containers were treated with 40 ml of the solution prepared. Concentrated Alga-root preparation (0.067 ml) was diluted in 350 ml of water and then, five plant containers were treated with 10 ml, five plant containers were treated with 20 ml and five plant containers were treated with 40 ml of the solution prepared. The average treatment rate for each preparation is the rate suggested by the manufacturer for commercial use. Three plant containers were used as controls. One week after auxins treatment, a nutrient solution was applied to each pot. This solution contained Urea [CO $(NH_2)_2$], monoammonium phosphate ($NH_4H_2PO_4$), and potassium sulfate (K_2SO_4) in a dose of 0.052 g, 0.034, and 0.034 g/kg of substrate, respectively. The dose of fertilizers corresponding to each pot was diluted into a volume of 100 ml of water and then applied. Seven weeks after planting, the substrate of all plant containers was treated with a sodium cyanide (NaCN) solution at a rate of 0.28 g/kg of substrate. NaCN was used to induce uptake of Au, Ag, and Cu and was chosen due to pH conditions of this substrate[18]. One week after treatment with NaCN, plants were harvested. Subsequently they were dried at 70° C in a drying oven for 12 h. Once dried, the plants were ground using a domestic dispositive Moulinex® at the Universidad Autonoma de Sinaloa and then prepared in the laboratory for analysis by atomic absorption spectrometry to determine the Au, Ag and Cu concentration in plants effected by each treatment[19]. Briefly, replicate subsamples of ground biomass (100 mg) were weighed into borosilicate test tubes and ashed for 20h at 500° C. The ash was then transferred into plastic digestion cups and digested on a water bath with aqua regia (5mL). Digest solutions were then made to10 mL volume with hydrochloric acid (2M), and analysed directly by flame atomic adsorption spectrometry.

RESULTS AND DISCUSSION

The objective of this study was to evaluate the influence of two auxins-based PGRs (rooting compounds) to improve Au, Ag, and Cu uptake by *H. annuus* plants grown on mine tailings amended with NaCN. However, before the effect of the PGRs could be quantified, the plants had to grow. We consider that a well-planned gold phytomining operation might consider important factors such as: the gold concentration in mineralized soil or waste mining; heavy metal and chemical contaminants in the substrate that may be toxic for plants; the pH level in the substrate; the chemical agent necessary to form a stable gold complex for uptake; the dose and time for application of the chelating agent[8]. Therefore, the physical-chemical properties of the substrate are a primary concern (**Table 1)**.

To increase the phytoavailability and/or translocation of heavy metals, the use of soil amendments has been suggested in literature[20]. However, the chemical selected in each case depends of some factors such as a pH level of the substrate. Wilson-Corral et al (2011) reported the effective use of NaCN as an amendment to induce gold uptake for tailings with a pH level near to 7.70. **Table 1** shows that the substrate for the current work was alkaline (pH = 7.8); a condition which favors the use of cyanide to form a stable soluble gold complex that can be taken up by plants18.

Considering the low concentrations of N, P, and K (1.82 and 217.33 mg/kg, respectively) shown in **Table 1**, a strategy of fertilization was designed to improve the fertility of the mine tailings for sunflower plants. All plants initially responded positively to fertilizers (increased growth rate relative to the control), however, after CN treatment, all plants showed signs of poor health. **Figure 1** shows that the average Cu concentration in the harvested plant tissues was very high (up to 486.57 mg/kg) and in our opinion is a function of the high copper concentration of the tailings used and the propensity for CN to solubilize Cu. It is probably the levels of Cu in the plant could have negatively affected plant health and may have limited the potential for gold uptake. For all Pro-root treatments (10, 20, and 40 ml of solution) the average Cu concentrations (486.57, 235.25, and 187.64 mg/kg, respectively) were higher than the average Cu concentrations in the controls (117.81 mg/kg). For the Alga-root treatment (10, 20, and 40 ml of solution) there was also an increase in the average Cu concentration relative to the control (357.18, 332.84, and 241.66 mg/kg, respectively), however there was no increase in Au concentration for any treatment. Review of scientific literature over the past 34 years shows that trace metal phytotoxicity follows the general trend (from most toxic to least toxic): Pb = Hg >Cu >Cd = As >Co = Ni = Zn >Mn. The median toxic concentration varies by about two orders of magnitude among the nine metals, being: 0.30 Pb, 0.47 Hg, 2.0 Cu, 5.0 Cd, 9.0 As, 17 Co, 19 Ni, 25 Zn, and 46 Mn [21].

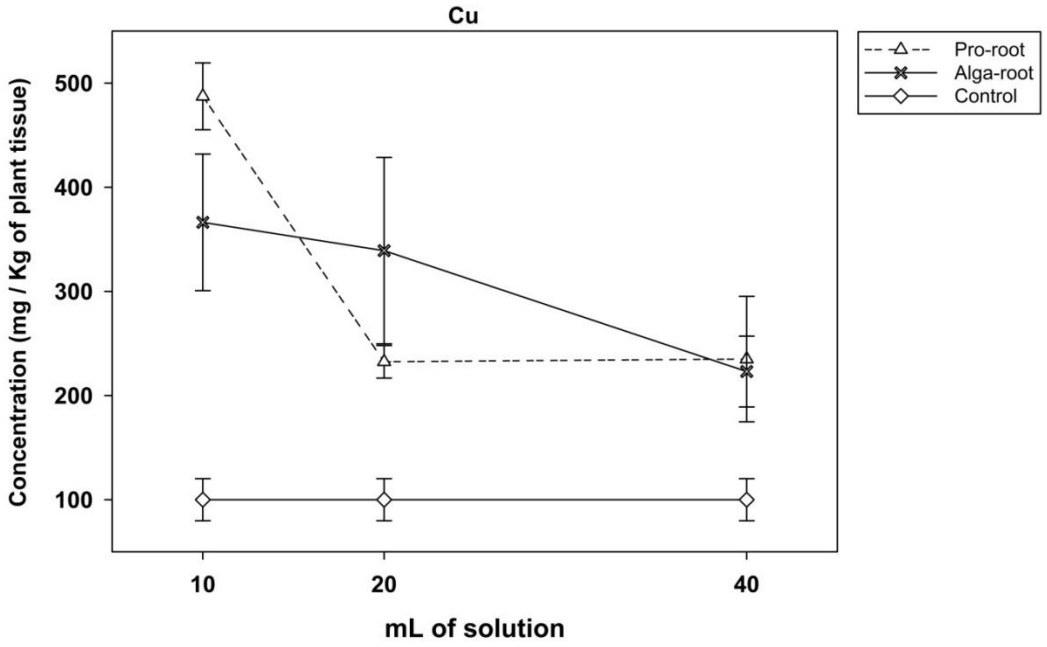

Figure 1. Copper concentration in plant tissues as a function of three doses of two plant growth regulators.

The highest average Au concentration in plant tissues corresponded to the treatment of 10 ml of the Pro-root solution. This was the only treatment to increase the Au concentration of the sunflowers relative to the control (**Figure 2**).

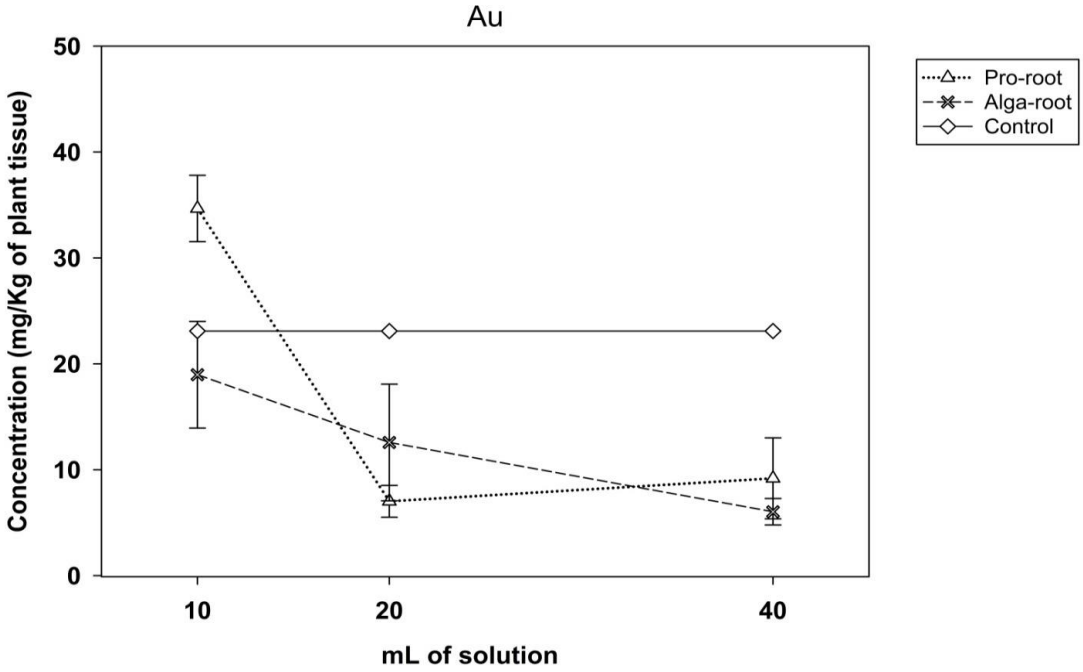

Figure 2. Gold concentration in plant tissues as a function of three doses of two plant growth regulators.

The effect of the PGRs on the Ag concentration in sunflower was less clear. **Figure 3** shows an increase in Ag concentration (relative to the control) for the 10 ml of Pro-root solution as well as the 10 and 20 ml of Alga-root solution (18.37, 22.48, and 12.03 mg/kg, respectively. The control concentration was 8.97 mg/kg.

Consideration of the data presented in **Figures 1**, **2** and **3** indicates that the Au, Ag and Cu concentration in plant tissues decreases as the dose of solution is increased for each of the treatments. Our interpretation is that an optimal dose of PGR can increase the average Cu concentration up to 313 %, the average Ag concentrations up to 150 %, and the average Au concentrations up to 36 % in plant tissues, relative to the control treatment of no PGR when *H. annuus* plant species is grown in mine tailings and treated with NaCN to induce the uptake of gold. This is the first study in which the potential benefits of plant growth regulators (auxins) to improve the Au, Ag and Cu concentration in plants grown at mine tailings are explored.

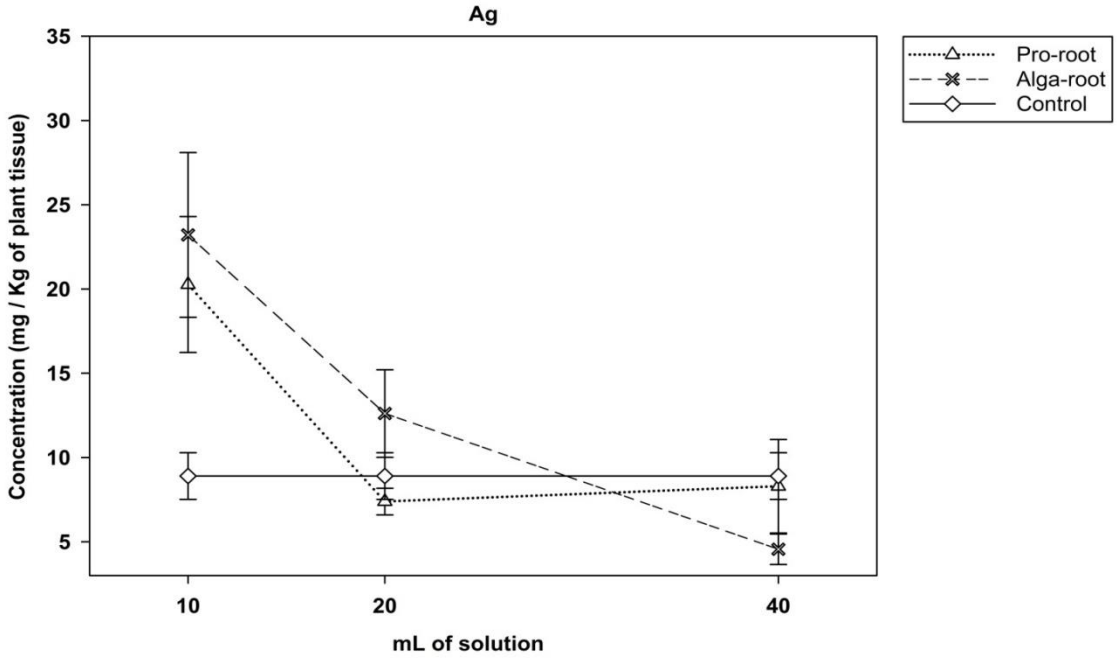

Figure 3. Silver concentration in plant tissues as a function of three doses of two plant growth regulators.

This study was developed considering that an improvement in the development of a plant's root system may increase the efficiency of Au phytoextraction to recover an economically viable amount of gold from the ground. Gold phytomining is a technique which can consider the full harvest of the plant. However, the Au concentrations not uniform throughout all organs of a plant[22]. A field trial developed by our research group in Mexico during the first months of 2013, showed that approximately 80 % of dry mater harvested is contained within the aerial parts of the plant while the root dry matter is approximately 20% of the total harvested biomass (unpublished study). At the same time the first and only report about the potentials effect of PGR on Au uptake by *B. juncea* plants was published[7]. In this study, plants were grown in a Hoagland's nutrient solution (50%). The results showed that a dose of 5 µM applied in the growth media of plants can induce Au concentrations in plant tissue above 190 mg/kg in the root dry biomass of *B. juncea*[23]. Interpreting the published data and considering the Au concentrations in leaves (75.2 mg/kg) and in roots (193.1 mg/kg) of *B. juncea*, the average Au concentration in the whole plant could be approximately 49 mg/kg dry weight [(0.2) (193.1) + (0.8) (75.2)]/2 = 49]. However, we should consider that the Hoagland's nutrient solution will not contain the high concentrations of toxic metals such as Cu that are contained in mine tailings, and therefore the possible interference on gold uptake described in our study may not have been apparent in this 2013 work. Moreover, the Au concentration in the hydroponic solution used as the growth media by Kulkarni and colleagues was 5.0 mg/kg. This Au concentration is over twice the concentration of the tailings we used in the current study, and the gold in the hydroponic solution will have been fully soluble. Therefore, results between the two studies are not directly comparable. However, both studies highlight that auxins can potentially enhance the absorption of Au when plants are grown in wastes from mining industry. Future studies should consider the possibility of developing more extensive laboratory trials to evaluate optimal mixes and doses of auxins to induce the maximum increases in Au, Ag, and Cu concentrations in plant tissues. Any increase in the concentration of these metals in plants harvested from phytomining operations will have a positive impact on the financial viability and profitability of the operation. The results we report in this paper, however, should be verified in the field before detailed economic assessment of PGR on gold phytomining can be considered.

CONCLUSIONS

We have shown that auxins applied to mine tailings during the second week of growth of *H. annuus* plant species, can improve the final Au, Ag and Cu concentration in plant tissue when the plants are treated with NaCN to induce precious metal uptake. Our study is in general agreement with the results of previous studies that have shown an increase in metal concentration in plants as a function of treatment with plant growth regulators. Future studies should define the optimal dose of auxins to maximize the potential gold concentration in plants grown for phytomining. Optimization trials should also evaluate the efficacy of a combination of endogenous plant growth regulators to induce increases in dry matter yields (root and shoot) as well as metal concentrations in plant tissues.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support with key PROFAPI2012/187, granted by the Programa de Fomento y Apoyo a Proyectos de Investigación (PROFAPI) of the Universidad Autonoma de Sinaloa for the development of this study. Moreover, thanks to Mr. K. D. Diepholz, Chairman of DynaResource Inc. as well as Dr. Jose Vargas Lugo, Operations Manager of DynaResource de Mexico S. A. de C. V. mining company for proving the necessary substrate from San Jose de Gracia Tailings Dam, to develop this trial.

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