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Gold phytoextraction and mining-degraded soil reclamation

Fitoextracciòn de oro y recuperación de suelos degradados por la minería

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Abstract

Gold phytoextraction is a technology used to obtain this metal (Au) throughout plants. Some of the benefits of this technology are the mitigation of the environmental impact, the fact that it does not imply the constant use of pollutants, and has the ability to promote soil reclamation. The aim of this research was to determine if *Brachiaria decumbens* has the potential to improve soil physical properties while had achieved a gold extraction. For this purpose, *Helianthus annuus* and *Brachiaria decumbens,* were used in a completely randomized design with four replicates. To induce gold uptake, ammonium thiocyanate was applied to plants. The statistical analysis was performed using the R-Studio software. Finally, it became evident that *B. decumbens* is a gold extractor plant, which also improves the soil physical qualities and compared to *H. annuus* and present higher concentrations of gold than the latter ($P<0.05$). The dose and mode of application of ammonium thiocyanate, modulates the gold absorption process since it causes toxicity in plants.

Keywords: Absorption, aggregates, transpiration, roots.

Resumen

La fitoextracción de oro es una tecnología que consiste en obtener este metal mediante el uso de plantas entre cuyos beneficios está que atenúa el impacto ambiental, no implica el uso constante de contaminantes y favorece la recuperación del suelo. El objetivo de este trabajo fue determinar si la *Brachiaria decumbens* tiene potencial para mejorar las propiedades físicas del suelo y para, a la vez, extraer oro del mismo. Otro objetivo fue corroborar si *Helianthus annuus*, además de ser fitoextractora de oro, tiene la capacidad de mejorar las propiedades físicas del suelo. Para esto se emplearon las plantas mencionadas en un diseño completamente aleatorizado con cuatro repeticiones. La absorción de oro fue inducida en las plantas mediante la aplicación de tiocianato de amonio y el análisis estadístico fue realizado usando el software R-Studio. Finalmente, se evidenció que *B. decumbens* es una planta auroextractora que, además, mejora las propiedades físicas del suelo al igual que *H. annuus*, presenta mayores concentraciones de oro que esta última ($P<0.05$). La dosis y la forma de aplicación del tiocianato de amonio modulan el proceso de absorción del oro al generar toxicidad en las plantas.

Palabras clave: Absorción, agregados, raíces, transpiración.

Introduction

Phytoextraction is a technology under development consisting of the utilization of metal-accumulating plants for metal extraction from polluted or metal-rich soils (Chaney *et al.,* 2007). This technique can be an alternative for metal extraction in zones where soil metal concentration is not enough for them to be extracted throughout conventional methods. Phytoextraction is suggested as a possible alternative not only to recover gold, but also nickel (Ni) and thallium (Ti) (Robinson, Bañuelos, Conesa, Evangelou & Schulin, 2009; Wilson-Corral, Anderson, Rodríguez-López, Arenas-Vargas & López-Pérez, 2011).

A hyperaccumulator plant is defined as a plant that accumulates heavy metals in concentrations one hundred times higher than any other plant could accumulate in the same substrate (Anderson, Brooks, Stewart & Simcock, 1998). In order to obtain a considered plant as a gold hyperaccumulator, it must present a concentration of 1mg.kg^{-1} (Brooks, Chambers, Nicks & Robinson, 1998; Harris, Naidoo, Nokes, Walker, & Orton, 2009).

The major interest in the usage of hyperaccumulator plants is the phytoremediation of contaminated areas by heavy metals. In addition to soil restoration, hyperaccumulation may represent an economic advantage in mining for some highly valuable metals, such as gold (Lamb, Anderson & Haverkamp, 2001). After accumulation, the plants are harvested and ashed to recover target metals. (Kulkarni *et al.,* 2013; Anderson, Moreno & Meech, 2005).

In 1998, Brooks, Chambers, Nicks & Robinson, called *phytomining,* the technique of using plants with phytoextraction abilities for mining purposes. This technique, consisting in the extraction of precious metals throughout plants, which has been recently studied especially for gold extraction (Anderson, Moreno & Meech, 2005; Girling & Peterson, 1980).

Despite having several reports on hyperaccumulator species, reports on capable plants of accumulating naturally gold do not exist (Harris *et al*., 2009). According to Lamb *et al*., 2001, a possible explanation is due to gold is present in the soil in forms in which is impossible to absorb throughout plants. However, Girling & Peterson (1980), reported that plants could actually absorb naturally gold. For instance, they found concentrations of 21.1 ppb of gold in stem samples of *Phacelia sericea* (Graham) A. Gray, which naturally developed in a substrate and presented a concentration of 1.6 ppb in a mining region in southern British Columbia, Canada.

Gold hyperaccumulation can be promoted by using chemical amendments, which have allowed to be in a form in which the plant can absorb the metal (Wilson-Corral *et al*., 2011). Among the most used chemical compounds are cyanide (CN^-), ammonium thiocyanate (NH_4SCN), and ammonium thiosulfate $(NH_4)_2S2O_3$. These compounds can be metabolized by soil microorganisms and later used as source of carbon, nitrogen and sulfur, according to Ebbs *et al*., 2010.

Chaney *et al.* (2007), mention two significant approaches for the development of phytoextraction technology as follows: the domestication of plant hyperaccumulators materials, the cloning of all genes needed for hyperaccumulation and tolerance to insert them and express them in a transgenic hyperaccumulator crop with high-biomass production. Therefore, is necessary an identification of high-metal-accumulator genotypes with fast growth, large potential of biomass production and a great response to nutrients (Bhargava, Carmona, Bhargava & Srivastava, 2012).

In this sense, Harris *et al.* (2009), mention the current interest in phytomining, which is mainly due to three aspects: a social pressure correlated to the environmental impact of conventional mining, the inability of current mining techniques to recover metals from substrates with low metal concentration in an economically acceptable way and the high cost of these metals in the present tense.

It is known that there are essentially two techniques to recover accumulated metal in soil biomass. The first technique is pyrolysis or combustion, which is followed by ash smelting. This technique is very attractive since the energy released can be used to produce electrical energy. The second technique is the acid digestion of the metal contained in plant biomass throughout electrowinning or solvent extraction to recover the metal (Harris *et al*., 2009). Other authors as Piccinin *et al.* (2007), show that phytomining is an economically viable technology. Some aspects to be considered for a successful phytoextraction are mentioned by Wilson-Corral *et al.* (2011).

Soils affected by mining present high levels of pollution due to a structure loss caused by soil aggregate destruction or the use of pollutants, which additionally affects the soil biota. These soils can be biologically stabilized and recovered throughout plant establishment with dense and deep root systems (Tordoff, Baker & Willis, 2000).

The plant establishment have allowed a soil protection against erosion while retains the soil particles due to an intervention roots to form a "network" that benefits the soil aggregation. This process restores the soil functional and structural attributes to the initial conditions previously to mining activity (Bronick & Lal, 2005). Moreover, a root secretion of exudates, facilitates the presence of fungi and bacteria from the rhizosphere, all of which, produce linking material which benefits flocculation process and soil particles aggregation. In fact, these processes enhance soil physical, chemical and biological properties (Angers & Caron, 1998; Bronick & Lal, 2005).

Due to loss of structure, mining-degraded soils present problems for the establishment of plant populations. This affects the soil porosity and infiltration, which limits the plant growth and root system development (Bengough *et al*., 2006). When occurs a loss in soil structure, one of the first effects that appears is soil compaction, which inhibits root growth and present a decreasing in water and nutrient uptake ability, which can lead to a poor plant development (Bronick & Lal, 2005).

Conversely, induced changes in soil structure are important due to an improvement in the water storage and its availability to retain soil water (Angers & Caron, 1998). Soil aggregation, promoted by plant establishment, is greater when a high root density and a long root length is present. Therefore, the use of fibrous and extensive roots had achieved a major impact on soil aggregation (Bronick & Lal, 2005).

An inappropriate mining extraction causes a negative impact in the environment and human health due to the use of pollutants in the beneficiation process. Some of those pollutants are mercury (Hg) and cyanide (CN^-). In the case of Hg, it accumulates in animals, and provokes air and soil pollution. In fact, mercury is harmful to humans in concentrations in the air as low as $1 \mu g.m^{-3}$ (Veiga, 2006).

In Colombia, the usage of mercury reaches amounts up to 150 $tons.year^{-1}$ (Veiga, 2008), which makes necessary the quest for other alternatives for gold extraction as phytoextraction, since is less risky, and also contributes to soil reclamation.

The aim of this research was to determine if *Brachiaria decumbens* Stapf and *Helianthus annuus* L. have the potential to improve soil physical properties while had achieved a gold extraction.

Materials and methods

The research was carried out at Universidad Nacional de Colombia, Medellin campus (UNALMED), in Antioquia, Colombia (6°15'47.07"N-75°34'40.02"O), with an altitude of approximately 1524 m.a.s.l., with an average temperature of 23°C, an average annual rainfall of 1397 mm and a relative humidity between 66% and 80%.

The substrate used corresponds to a soil sample from two mining zones in the department of Antioquia, Colombia as follows: The municipalities of Gómez Plata and Santa Fe de Antioquia, Colombia. These two soil samples were homogenized and chemically characterized in the Soil Analysis Laboratory of UNALMED, Medellin-Antioquia, Colombia.

The species used were sunflower (*H. annuus)* and Brachiaria (*B. ducumbens),* which were sown in containers with 2 kg of substrate, and they were fertilized with 30 g of vermicomposting.

Experimental design for gold phytoextraction

The experimental design corresponded to a completely blocks randomized with six treatments and four replicates, where was carried out a total of twenty-four experimental units as follows: three treatments with sunflower, and the other three with Brachiaria with an addition of ammonium thiocyanate (NH_4SCN) to the substrate in doses of 1.24 and 2.48 g kg^{-1}, respectively and a treatment without ammonium thiocyanate addition. Therefore, ammonium thiocyanate was applied in a solution with 100 ml of distilled water in each experimental unit ten days previous to harvest. The experiment was established in fifty-two days.

Gold content determination in plants and substrate

Substrate

The soil sample was analyzed in the Laboratory of Mineralogy Institute CIMEX of UNALMED, Medellin-Antioquia, Colombia. Gold concentration was determined throughout atomic absorption spectrophotometry (device: ICE 3000 SERIES AA, Espectromole Thermoscientific). Gold concentration in soil sample was 1.2 ppm (Table 1).

Table 1. Soil physicochemical characterization used for phytoextraction evaluation

Parameter	Value	Units	Method of extraction	Analytical determination
Au	1.2	$mg.kg^{-1}$	Aqua regia digestion	Atomic absorption
pH	6.5	-	H_2O (1:1, V:V)	Potentiometry
Ca^+	11.2	$Cmolc.kg^{-1}$	Ammonium acetate 1*M*	Atomic absorption
Mg^+	1.5	$Cmolc.kg^{-1}$	Ammonium acetate 1*M*	Atomic absorption
P	28	$mg.kg^{-1}$	Bray II (NH_4F 0.03 *M* y HCl 0.1 *M*)	Spectrophotometry
S	286	$mg.kg^{-1}$	$Ca(H_2PO_4)_2$ 0.008 *M*	Turbidimetry
Fe	61	$mg.kg^{-1}$	Olsen ($NaHCO_3$ 0.5 *M*)-EDTA	Atomic absorption
Mn	10	$mg.kg^{-1}$	Olsen ($NaHCO_3$ 0.5 *M*)-EDTA	Atomic absorption
Cu	21	$mg.kg^{-1}$	Olsen ($NaHCO_3$ 0.5 *M*)-EDTA	Atomic absorption
Zn	3	$mg.kg^{-1}$	Olsen ($NaHCO_3$ 0.5 *M*)-EDTA	Atomic absorption
B	0.2	$mg.kg^{-1}$	Hot water	Spectrophotometry
Organic matter	1.2	%	Walkley & Black	Redox titration
Sand	68	%		
Silt	24	%		
Clay	8	%		
True density	2.95	$g.cm^{-3}$		PENTAPYC 5200e

Plants

Ten days after the chemical amendment (NH_4SCN) was applied, the twenty-four experimental units were harvested, washed and finally dried in an oven at 105°C until constant weight was reached. The resulting dried material was grinded for subsequent analysis by atomic absorption spectrophotometry, which have allowed the determination of gold concentration in each experimental unity. A general diagram of the procedure carried out for phytoextraction is shown in Figure 1.

Figure 1. Procedure carried out for gold phytoextraction. Substrate homogenization (A). Substrate disposition (B). Application of vermicomposting (C). Phytoextractor plants (D). Grinder (E). Atomic absorption spectrophotometer (F).

Statistical analysis

A Shapiro Wilk test and Bartlett test, were performed to evaluate the data normality and variances homogeneity. Specifically, those data that violated the ANOVA assumptions, a nonparametric test Kruskal-Wallis (α = 0.05), was carried out for gold concentration with a Wilcoxon test (the pairwise.wilcox.test function) without *p* adjusted (α = 0.05) to identify significant difference among treatments and statistical significance for all comparisons was made at $p<0.05$. R-Studio software ® was used to compare the mean values of treatments.

Soil physical properties

A changes evaluation of soil physical properties in the substrate due to the presence of *B. ducumbens* and *H. annus,* was performed 45 days after sunflower and brachiaria were sown, respectively. The soil indicators used were as follows: bulk density, measured with a bulk density ring (IGAC, 2004), true density throughout an automatic density analyzer (PENTAPYC 5200e, Quantachrome Instruments, Florida USA), and soil aggregates, which were weighted and the average diameter were measured after wet screening (WADAWS) according to De Lehner & De Boot method, modified by IGAC (2006).

Results

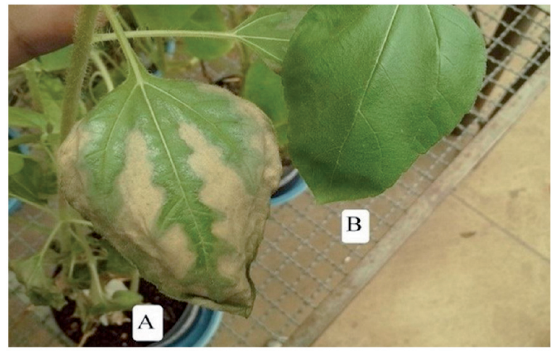

Figure 2. Evidence of toxicity in leaf blade caused by ammonium thiocyanate (NH_4SCN). With toxicity (A). Without toxicity (B).

B. decumbens and *H. annuus* showed the effect of plants in soil structure. In both species, bulk density decreased in 1 g.cm^{-3}, and weighted average diameter after wet screening (WADAWS) increased in an average of 250 µm (Figure 3).

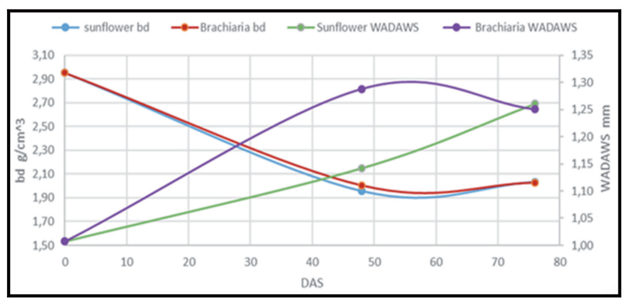

Figure 3. Change in physical variables (Bd = bulk density, WADAWS = weighted average diameter after wet screening) evaluated in the substrate with sunflower (*Helianthus annuus* L.) and the substrate with Brachiaria grass (*Brachiaria decumbens*). DAS: days after sowing.

It should be taken into account that this indicates an improvement in soil physical properties due to the action of system root in the soil. In fact, an improvement in the substrate was also evident since the initial substrate had not structure (fine grain) (Figure 1A), compared to the final substrate which presented united particles, which formed a structure (Figure 4).

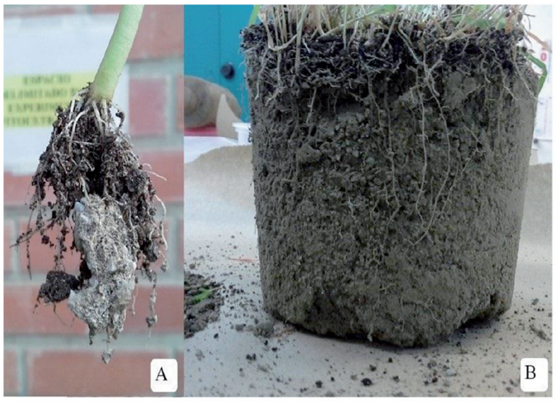

Figure 4. Evidence of phytostructute in soil generated by the action of sunflower (*Helianthus annuus*) (A) and (B) Brachiaria grass (*Brachiaria decumbens)*

Given these concerns, the treatment which presented greatest gold accumulation in dry material was Brachiaria 2.48, which had achieved a concentration of 0.05 $mg.kg^{-1}$. In the other hand, the treatment with lowest gold accumulation was Sunflower 0, with 0 $mg.kg^{-1}$. The maximum absorption rate was of 0.09 $mg.kg^{-1}$ in B2.48, followed by B1.24 with 0.05 $mg.kg^{-1}$ (Table 2).

Table 2. Gold concentration in *B. decumbens* and sunflower *H. annus,* under different concentrations of NH_4SCN.

Treatment	Average gold concentration mg.kg^{-1} of dm	Standard deviation	Maximum gold concentration **mg.kg^{-1} of dm**
Brachiaria 0	0.0175	0.0206	0.04
Brachiaria 1.24	0.0475	0.0231	0.05
Brachiaria 2.48	0.05	0.0263	0.09
Sunflower 0	0	0.0000	0.00
Sunflower 1.24	0.00275	0.0035	0.008
Sunflower 2.48	0.00075	0.0015	0.003

Treatments with different concentrations of NH_4SCN: Brachiaria 0; 0 $g.kg^{-1}$; Brachiaria 1.24: 1.24 $g.kg^{-1}$; Brachiaria 2.48; 2.48 $g.kg^{-1}$; Sunflower 0;0 $g.kg^{-1}$; Sunflower 1.24, 1.24 $g.kg^{-1}$; Sunflower 2.48, 2.48 $g.kg^{-1}$ of soil. Where *dm* stands for dry material accordingly to Kruskal-Wallis test (α = 0.05).

Table 3, shows significant differences among treatments. In fact, throughout Wilcoxon test was found that B1.24, was significantly different from treatments with sunflower and B0 $g.kg^{-1}$, but was not significantly different from B2.48. Despite having a higher mean B2.48 than B1.24, B2.48 was not significantly different from all treatments. This could be due to high dispersions on B2.48 data. A possible explanation for this is based on a plant toxicity, which translates into a quickly death plant (Table 2, Figure 5).

Table 3. Kruskal-Wallis test (α = 0.05).

Kruskal-Wallis test	
Chi-square	13.371
df	5
p-value	0.02014

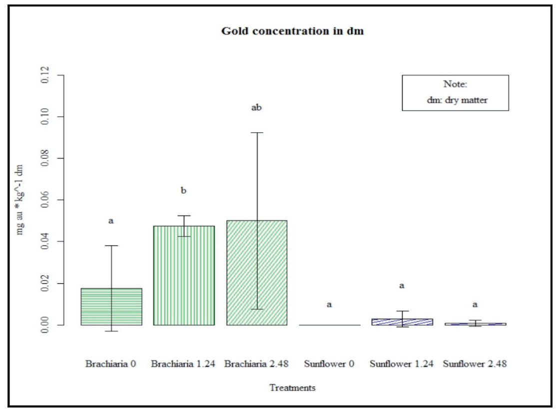

Figure 5. Average gold extraction in $mg.kg^{-1}$ of dm per treatment.

Same letters indicates there are not significant differences. Therefore, different letters indicates significant differences. 0, 1.24, 2.48 grams of ammonium thiocyanate per kg of soil.

Discussion

Compared to gold concentration values of 1.64-55.6 $mg.kg^{-1}$ in sunflower *Helianthus annuus* L., and 5-76 $mg.kg^{-1}$ in mustard, *Brassica juncea* (L.) Czern., reported by Wilson-Corral *et al.* (2011), and Lamb *et al.* (2001), gold concentration values obtained in this research were very low, since the highest concentration in this experiment was of 0.09 $mg.kg^{-1}$ in *B. decumbens.* This could be due to a strong toxicity in plants treated with NH_4SCN, which generated necrosis in the leaf blade (Figure 2).

Nonetheless, it is worth noting that the substrates used in this research, presented a higher gold concentration (2.35 $mg.kg^{-1}$ and 5 $mg.kg^{-1}$, respectively), which is according to previously reported by Wilson-Corral *et al.* (2011), where is considered as one of the factors to be taken into account for a successful gold extraction.

The application form of NH_4SCN could have disfavored the physiological mechanisms which have allowed the plant to absorb gold. Conversely, dose applications at different timing or in lower doses, which confirmed the reported data and obtained by Anderson *et al.* (1998), could increase the plant ability to absorb gold from soil in a more effective way and during a longer period of time due to a lower toxicity, which could lead to a greater metal absorption.

This drawback can be overcome by potential of *B. decumbes* acting as gold extractor, perhaps related to a genetic condition and due this occurs in high density planting. This type of grass is constituted with a large transpiratory surface compared to sunflower, which have allowed a great movement of gold released on the soil solution throughout xylematic continuum.

Conclusion

Brachiaria decumbens has potential for gold phytoextraction, presenting a maximum extraction value of 0.09 mg.kg^{-1} of dm, which is higher than 0.008 mg.kg^{-1} found on sunflower *Helianthus annuus* L., a species with reported potential for gold phytoextraction. The application of ammonium thiocyanate are thought to contribute and modules the gold absorption process since produces a plant toxicity, which is more evident in the leaf blade death in both species. Alternatively, is believed to be an outcome of hampering transpiration and gold uptake from the substrate. Both species, *Brachiaria decumbens* Stapf. and *Helianthus annuus* L., had achieved a soil physical properties improvement with an increasing soil aggregation and soil weighted average diameter after wet screening (WADAWS) with an average of 250 µm. Therefore, both species also contributes to a decreasing soil bulk density (Bd) with an average of 1 g.cm^{-3}.

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