Ecotoxicology and Environmental Safety 154 (2018) 180–186

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Induced accumulation of Au, Ag and Cu in *Brassica napus* grown in a mine tailings with the inoculation of *Aspergillus niger* and the application of two chemical compounds[☆]

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ARTICLE INFO

Keywords:
Hyperaccumulation
Phytomining
Plant biomass
Chemical lixiviation
Precious metals

ABSTRACT

This study evaluated the ability of *Brassica napus* for extracting gold (Au), silver (Ag) and copper (Cu) from a mine tailings, with the inoculation of two *Aspergillus niger* strains, and the application of ammonium thiocyanate (NH_4SCN) or ammonium thiosulfate [$(NH_4)_2S_2O_3$]. After seven weeks of growth inoculated or non-inoculated plants were applied with 1 or 2 g kg^{-1} of either NH_4SCN or $(NH_4)_2S_2O_3$, respectively. Eight days after the application of the chemical compounds, plants were harvested for determining the total dry biomass, and the content of Au, Ag, and Cu in plant organs. Application of $(NH_4)_2S_2O_3$ or NH_4SCN resulted in enhanced Au-accumulation in stems (447% and 507%, respectively), while either $(NH_4)_2S_2O_3$ + *Aspergillus*, or NH_4SCN increased the Au-accumulation in roots (198.5% and 404%, respectively) when compared to the control. Treatments with $(NH_4)_2S_2O_3$ or $(NH_4)_2S_2O_3$ + *Aspergillus* significantly increased ($P \leq 0.001$) the accumulation of Ag in leaves (677% and 1376%, respectively), while NH_4SCN + *Aspergillus*, and $(NH_4)_2S_2O_3$ enhanced the accumulation in stems (7153% and 6717.5%). The Ag-accumulation in roots was stimulated by NH_4SCN + *Aspergillus*, and $(NH_4)_2S_2O_3$ + *Aspergillus* (132.5% and 178%, respectively), when compared to the control. The combination of NH_4SCN + *Aspergillus* significantly enhanced the Cu-accumulation in leaves (228%); whereas NH_4SCN + *Aspergillus*, or $(NH_4)_2S_2O_3$ + *Aspergillus* resulted in greater accumulation of Cu in stems (1233.5% and 1580%, respectively) than the control. Results suggest that either NH_4SCN or $(NH_4)_2S_2O_3$ (with or without *Aspergillus*) improved the accumulation of Au and Ag by *B. napus*. Accumulation of Au and Ag in plant organs overpassed the hyperaccumulation criterion (> 1 mg kg^{-1} of plant biomass); whereas Cu-accumulation in stems and roots also overpassed such criterion (> 1000 mg kg^{-1}) by applying either NH_4SCN or $(NH_4)_2S_2O_3$ + *A. niger*.

1. Introduction

Mining industry annually generates significant amounts of residues that are accumulated worldwide. These residues are generated due to mineral grinding after recovering precious metals by conventional processes (Wang et al., 2014). Zacatecas state (Mexico) has the most important mine industries for extracting gold (Au), silver (Ag), and copper (Cu) (González-Valdez et al., 2016); furthermore, extraction of Au and Ag is mainly based on cyanide lixiviation (Trapp et al., 2003). However, in spite of the development of mining industry, Au is not

[☆] Capsule: The combination of chemical leaching agents with the inoculation of two *Aspergillus* strains improved the phytoaccumulation of Au, Ag and Cu in *Brassica* plants.

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https://doi.org/10.1016/j.ecoenv.2018.02.055
Received 21 September 2017; Received in revised form 9 February 2018; Accepted 15 February 2018
Available online 22 February 2018
0147-6513/

completely recovered by means of traditional technologies (Wilson-Corral et al., 2012); then, abundant quantities of this metal (5 mg kg^{-1}) may keep retained in waste dumps mines (Kulkarni et al., 2013). Traditional techniques for recovering Au and Ag are highly expensive and harmful to the environment, generating potentially toxic elements (PTE) such as lead (Pb), cadmium (Cd), arsenic (As), thallium (Tl), and mercury (Hg), or other toxic compounds like cyanide (CN), that accumulate and persist in soils (Rodríguez et al., 2007). The later represents a constant risk potential for ecosystems and human health.

Due to the negative environmental impacts of traditional techniques for recovering either Au or Ag (Rodríguez et al., 2007; Trapp et al., 2003), alternate processes have been proposed, for instance, phytomining which is based on the utilization of plants to absorb and accumulate precious elements in their organs (Gardea-Torresdey et al., 2005). Phytomining is addressed to those sites whose mining residues are not economically suitable for applying conventional methods to recover residual metals (Wilson-Corral et al., 2011). This biotechnology has been utilized for recovering precious metals like gold, silver, platinum, nickel, and palladium (Rodríguez et al., 2007).

Metal-hyperaccumulator plants may accumulate more than 1 mg of Au or Ag per kg of dry matter (Anderson et al., 1998; Pratas et al., 2013), or 1000 mg Cu kg^{-1} (Malaisse et al., 1978; Pratas et al., 2013). Examples of plant species used for Au-phytomining purposes are *Helianthus annuus* L. (Wilson-Corral et al., 2011), *Zea mays* L., *Brassica juncea* L. (Anderson et al., 2005), *Chilopsis linearis* (Cav.) Sweet (Gardea-Torresdey et al., 2005). Plants reported as Ag-accumulators are *Mentha suavolens* Ehrh., and *Rubus ulmifolius* Schott (Pratas et al., 2013), whereas *Aeolanthus biformifolius* De Wild. Malaisse et al. (1978) is reported for Cu-accumulation.

Brassica napus L. (Brassicaceae) may accumulate significant amounts of PTE in its abundant biomass. This plant species is proposed for phytoextraction strategies due to its ability for growing at highly perturbed sites with metals (Zaier et al., 2010; González-Valdez et al., 2016).

Both Au and Ag metals are present in soil as insoluble forms (Sheoran et al., 2009). Thus, induced hyperaccumulation processes are used for solubilizing metals, and for favoring metal availability and uptake for plants (Piccinin et al., 2007). Application of chemical compounds [NH_4SCN or $(NH_4)_2S_2O_3$, for instance], and microbial inoculation may induce the lixiviation or the solubilization of Au, Ag, or Cu in soils; then, favoring metal uptake and accumulation in plant organs (Msuya et al., 2000; Southam et al., 2009; Wilson-Corral et al., 2011; Maluckov, 2015).

Few studies demonstrated that the inoculation of *Aspergillus niger* in solutions with high concentrations of cyanide, promotes the recovery of 57% of Au, 100% of Ag, and 55% of Cu (Gomes et al., 1996). Kapoor and Viraraghavan (1997) showed that the removal of Pb, Cd, and Cu from aqueous media is enhanced by inoculating *A. niger*. This fungal species solubilizes and mobilizes Cd, Cr, Cu, Zn, Ni, and Pb from solid substrates (Brandl and Faramarzi, 2006). In the case of metal recovery from electronic wastes, the combined inoculation of *A. niger* and *Penicillium simplicissimum* resulted in induced removal of 65% Cu and Sn, and 95% Al, Ni, Pb, and Zn (Brandl et al., 2001). Moreover, the single inoculation of *A. niger* MxPE6 enhanced the Au-lixiviation (42%); whereas its combination with *A. niger* Mx7 the metal lixiviation was of 87% and 29% for Au and Cu, respectively (Madrigal-Arias et al., 2015). In contrast, the maximum lixiviation of metals achieved from mining residues due to *Aspergillus* inoculation was about 68%, 46%, and 34%, for Cu, Zn, and Ni respectively (Mulligan et al., 2004). The use of *A. niger* in combination with cyanide (181.6 mg L^{-1}) resulted in improved removal of Au, Ag, Cu, Fe, and Zn from highly-rich metal leachates (Gomes et al., 1998).

In spite of the information previously described, little is known about the interaction of strains of *A. niger* with the combination of chemical compounds to enhance the efficiency of *B. napus* for phytoextracting precious metals from mine tailings. Thus, this study evaluated the capability of *B. napus* to phytoextracting Au, Ag, and Cu from a mine tailings, due to the application of NH_4SCN or $(NH_4)_2S_2O_3$, and the combined inoculation of two strains of *Aspergillus niger* MxPE6 + *Aspergillus niger* Mx7.

2. Materials and methods

2.1. Sampling site, recollection, and analysis of the mine tailings

The mine "El Bote" is located at Guadalupe City (22° 46′ 55.54″ N and 102° 35′ 19.22″ O, at 2398 masl, average temperature 16 °C, and average annual precipitation of 510 mm), in Zacatecas sate (Mexico) (González-Valdez et al., 2016; PMEZ, 2013). The mining industry in Zacatecas was set for more than 450 years (González-Valdez et al., 2016), and during 2012 generates an average production of 21,678 t for Au, 2,333,653 t for Ag, and 51,262 t for Cu (PMEZ, 2013).

Three composed samples were taken from the mine tailings (10 kg per sample) from two sites separated 30 m each other, and considering the collection of the residue at 0–30 cm of depth (NMX, 2006). Samples were labelled and transported to the laboratory in plastic bags hermetically sealed. The mine tailings was set on plastic trays for allowing dryness at room temperature (25 °C) for 96 h, then, sieved through mesh of 2 mm diameter to allow homogeneity of size particles for further chemical analyses. Chemical analyses consisted on determining organic matter content (Walkley and Black, 1934), pH (1:2H_2O) and electrical conductivity (1:2H_2O) (SEMARNAT, 2001; Haluschak, 2006). The total content of Ca, Mg, Na, K, Cl, P, B, CO_3, HCO_3, and SO_4 was performed by standardized methods described by SEMARNAT (2001) and Haluschak (2006). The total content of Au, Ag, and Cu was determined by ICP-AES in accordance to the EPA method (USEPA, 2007). Table 1 shows the content of target metals as well as the values of physical and chemical parameters in the mine tailings.

2.2. Experiment establishment

This research was conducted under greenhouse conditions during 57 days. Average maximum and minimum temperature was 36 °C and 15 °C, respectively, and average maximum and minimum relative humidity was 78% and 36%, respectively (Data logger Hobo series H8).

Seeds of *B. napus* were sown in polystyrene trays with 200 cavities

Table 1
Physical, chemical properties and content of three metals (Au, Ag, and Cu) in a mine tailings collected at El Bote, Zacatecas (Mexico).

Parameter	Value	Parameter	Value	Parameter	Value
Au	0.5164 ± 0.001 mg kg^{-1}	CO_3	1.92 meq L^{-1}	EC	0.33 dS m^{-1}
Ag	22.1 ± 4.7 mg kg^{-1}	HCO_3	0.16 meq L^{-1}	Organic matter	0.28%
Cu	107.0 ± 10.3 mg kg^{-1}	Cl	0.8 meq L^{-1}	Na	0.27 mg kg^{-1}
SO_4	0.54 meq L^{-1}	K	0.12 mg kg^{-1}	B	0.55 mg kg^{-1}
Ca	1.68 mg kg^{-1}	P	11.13 mg kg^{-1}	Mg	1.05 mg kg^{-1}
pH	8.5				
Clay	91.4%	Loam	5.2%	Sand	3.4%
Texture	Clay				