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CEPAS NATIVAS TOLERANTES A METALES PESADOS AISLADOS DEL PASIVO MINERO DE LA MINA HUALILÁN, ARGENTINA

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PALABRAS CLAVE
Hongos filamentosos
Cepas nativas
Pilas de lixiviación
Metales pesados

RESUMEN

La contaminación por metales pesados es actualmente uno de los problemas ambientales más serios y complejos de tratar. Las pilas de lixiviación de las industrias mineras se caracterizan por su elevado contenido en metales disueltos, además de ser considerado un hábitat propicio para el desarrollo de microorganismos resistentes. Entre ellos, aquellos microorganismos autóctonos con adaptaciones fisiológicas que le permiten tolerar elevadas concentraciones de metales pesados, los cuales son considerados como candidatos para procesos de biorremediación. Por esto, el objetivo de este estudio fue aislar y seleccionar cepas de hongos filamentosos con potencialidad para la remoción de metales pesados. Se tomaron muestras de pilas de lixiviación de la Mina Hualilán en la provincia de San Juan (Argentina). El aislamiento de los hongos filamentosos se llevó a cabo en medio Sabouraud-glucosa-agar con antibióticos y se identificaron por técnicas de biología molecular (amplificación y secuenciación del fragmento ADNr 18S y 26S, ITS1, ADNr 5.8S e ITS2). Se estudió la tolerancia a Cu (II) y Pb (II) en placas con medio Agar papa dextrosa (PDA) con concentraciones entre 25 y 800 ppm de cada metal. Las condiciones de incubación fueron a 28 ºC durante un período de total desarrollo de los hongos comparado con una placa control (sin metal), midiendo diámetros de crecimiento. Se realizó un análisis estadístico haciendo un análisis de varianza y prueba de comparación de promedios Duncan, con el programa estadístico Anova multifactorial bajo un diseño experimental completamente al azar con arreglo factorial 2x6x1, una caja de Petri como unidad experimental, y tres repeticiones. Como resultado se aislaron tres cepas: *Penicillium simplicissimum*, *Fusarium sp* y *Penicillium funiculosum*. Los hongos identificados presentan tolerancias a distintas concentraciones de soluciones de los metales, donde la cepa más tolerante fue *Penicillium simplicissimum*.

HEAVY METALS TOLERANT NATIVE STRAINS ISOLATED FROM MINING WASTE OF THE HUALILÁN MINE, ARGENTINA

KEYWORDS
Filamentous fungi
Native strains
Leaching piles

ABSTRACT

Heavy-metal contamination is one of the most serious and complex environmental problems to be dealt with nowadays. Leaching piles from the mining industry are characterized by a high content of

Heavy metals	dissolved metals. In addition, they are considered to be a suitable habitat for the development of resistant microorganisms. Among such microorganisms are those native ones that are physiologically adapted to tolerate high concentrations of heavy metals and thus, are considered to be candidates for bioremediation processes. Taking this into consideration, the aim of this study was to isolate and select filamentous fungal strains with the potential to remove heavy metals. Samples of leaching piles were drawn from Hualilán Mine in the province of San Juan (Argentina). Isolation of filamentous fungi was carried out in a Sabouraud-glucose-agar medium with antibiotics, and the fungi were identified by using molecular biology techniques (amplification and sequencing of the fragment ADNr 18S y 26S, ITS1, ADNr 5.8S e ITS2). Tolerance to Cu (II) and Pb (II) was studied in plates with Potato dextrose agar (PDA) medium with concentrations between 25 and 800 ppm of each metal. Incubation conditions were at 28 °C during the period of total development of the fungi and compared with a control plate (without metal) by measuring growth diameters. A statistical analysis was carried out through variance analysis and Duncan means comparison test, with the ANOVA multifactor statistical software under a completely randomized experimental design with 2x6x1 factorial arrangement, a Petri dish as an experimental unit, and three repetitions. As a result, three strains were isolated, namely, *Penicillium simplicissimum, Fusarium sp* and *Penicillium funiculosum*. The fungi identified show tolerances to different concentrations of metal solutions, the most tolerant strain being *Penicillium simplicissimum.*

1. INTRODUCTION

Heavy metal contamination is one of the most serious environmental problems nowadays. Industries such as mining, metal smelting, fuel and energy production from petroleum, the fertilizer and pesticide industry and their applications, among others, produce waste that contains high concentrations of heavy metals. These effluents are dumped into the environment and distributed in the soil, water and air. Heavy metals are not chemically or biologically degradable like most organic compounds; therefore their accumulation in nature is highly dangerous. The occurrence of heavy metals affects biological diversity and cause serious ecological problems such as the increase of bioaccumulation and the magnification of toxins in the food chain, resulting in several diseases such as lungs and digestive tract irritation and/or cancer, low growth rates in plants, death of animals and others health alterations [1].

During the excavation and extraction of minerals in the mining activity and, depending on both, the characteristics of the mining method (either underground or open-pit), and the geological characteristics of the site, there is an important movement or displacement of land and rocks [2]. The ore extracted from the mine is subjected to a process of benefit and concentration that results in the generation of a large amount of waste or fragments of the mineral that sometimes contain a significant amount of metals or residues of the chemical used in the concentration. In the concentration stage of the mineral, chemical reagents, in particular, cyanide and sulfuric acid are frequently used, the residues of which remain in the fragments of the mineral that is discarded. The contact of the rain with this waste produces contamination of the draining water [3].

It is known that heavy metal-tolerant microorganisms, which generally coincide with those that have been isolated from areas contaminated by those heavy metals [4, 5] are usually good candidates to be studied and applied in biological removal technologies as a sustainable alternative of lower impact on the environment. Various types of tests exist such as strength, tolerance and Minimum Inhibitory Concentration (MIC) that are used to select the best strains when heavy metal dissolved ions have to be removed. These tests are intended to determine whether the strains are able to grow in an environment contaminated with such metals. Tolerance tests, in turn, clearly establish a comparison between the potential ability to grow in a contaminated environment and the natural ability to grow [6].

In the area of microbial ecology, there is an increased interest in the study of microbial diversity and the dynamics of its populations. This helps us deepen our knowledge both on the composition of the communities existing in contaminated soils and on their evolution during the pollutants removal processes. It also helps determine which microorganisms are capable of adapting to and exploring contaminated habitats [7]. By means of molecular techniques, the structure and composition of the microbial communities associated to leaching piles was studied in order to select tolerant microorganisms.

Fungi are one of the main components of biota in soils and mineral substrates. Also, under certain environmental conditions, they may be efficient biogeochemical agents and metal bioaccumulators. Among them, *Penicillium sp*. is one of the most prominent genera in this methodology [8]. Fungi under stress may adapt to the above conditions either through a temporary alteration in their development patterns or through changes in their physiological characteristics, depending on the toxicity of metals, which in turn is influenced by the existing concentration of the metal [9].

Taking the above facts into consideration, this work describes the isolation of native strains of microorganisms present in waste from the Hualilán Mine of the Province of San Juan, Argentina in order to identify and select those species tolerant to heavy metals.

2. EXPERIMENTAL METHODOLOGY

2.1. Sampling, chemical analysis and visual identification of minerals of the solid

The samples were collected from 10 different points in the leach pile (waste material) from the Hualilán mine, province of San Juan (Argentina), an abandoned mine where predominant elements such as gold and silver were extracted together with subordinate elements such as copper, zinc and lead (Figure 1).

Sampling was performed manually by digging with shovel and approximately 500 g of sample was removed from each selected point. The material used was previously sterilized and the samples were stored in sterile plastic containers and kept at 4 °C temperature before being processed [10].

Chemical analysis of a representative sample of the leach pile material was carried out by the instrumental analysis laboratory of the Mining Research Institute (IIM), determined by Inductively Coupled Plasma (ICP-OES Perkin Elmer 7300DV). In order to determine the mineral phases present in the sample, a steroscopic microscope Leica S6D was used.

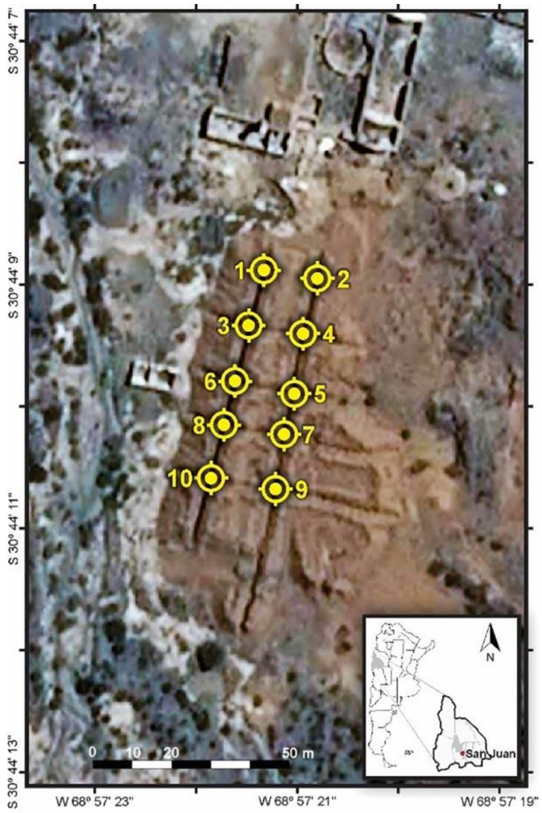

Figure 1. *Hualilan Mine, Argentina, Satellite photo.*

2.2. Isolation of filamentous fungi

Heavy metal-tolerant filamentous fungi were isolated using Sabouraud glucose agar (Biopack) as culture medium, the composition of g/L is: animal digestion pancreatic tissue (5), casein pancreatic digestion (5); dextrose anhydrous (36.4) and bacteriological agar (15).

A portion of 1 g of mining waste was weighed and sterile distilled water was added up to a final volume of 10 mL in a test tube and then stirred until homogenization. Aliquots of 0.1 mL-aliquots were withdrawn, serially diluted and placed on plates on Sabouraud glucose medium, in triplicate. Plates were incubated at 28 ºC for 24 h, and observed regularly to monitor growth development. Filamentous fungi presenting different colony morphotypes were isolated and mantained in the same agar medium at 5 °C.

2.3. Identification of the isolations

For the molecular biology identification; the selected fungi were individually grown in a Sabouraud Dextrose medium at 30 °C for 72 h in an orbital shaker (250 rpm). The mycelia were then collected by centrifugation at 10000 × g (10 min), suspended again in 2 M NaCl and finally washed twice with sterile distilled water. The washed mycelial mats were frozen in liquid nitrogen and ground to powder by using a sterile pestle and mortar. The DNA from the ground mycelia were immediately extracted with phenol: chloroform: - isoamyl alcohol (25:24:1) and washed twice with chloroform: - isoamyl alcohol (24: 1). Next, two volumes of absolute ethanol and 0.1 volume of 3M potassium acetate were added to the final aqueous phase in order to achieve DNA precipitation, mixed by inversion and then centrifuged (8000 ×g, 10 min, 4 °C). The pellet was washed twice with 70 % (in weigt) ethanol, dried and finally suspended again in sterile water. Sequencing of the genes nucleotides was performed by Macrogen (Korea). The sequences were analyzed and edited when necessary by using the Invitrogen Vector NTI Advance 10.3.0 software (Invitrogen, San Diego, CA, USA). The identification of the strain was carried out by comparison with the sequences of strain-type available in MycoBank by using the BLAST tool. The sequences were also compared with the species hypothesis in the UNITE 13 database [11-13].

Macroscopic identification was performed according to a taxonomic scheme using MEA (in g/L: malt extract 30, peptone from soybean 3, bacteriological agar 15), CYA (in g/L: K_2PO_4 1, czapeck concentrate 10, yeast extract 5, sacarose 30, agar-agar 15 and traces of metal 1ml/L), AGS (in g/L: animal digestion pancreatic tissue 5, casein pancreatic digestion5; dextrose anhydrous 36.4 and bacteriological agar 15), PDA (Potato Dextrose Agar, Biopack in g/L: potato extract 4; glucose 20; bacteriological agar 15), and G25N (K_2HPO_4 1 g/L, czapeck concentrate 10 ml/L) as culture mediums [14,15].

2.4. Heavy metal ions tolerance

For this test, a PDA (Potato Dextrose Agar) commercial culture medium Biopack was used, the composition of which is: potato extract (4 g/L); Glucose (20 g/L); bacteriologic agar (15 g/L). After being sterilized in autoclave at 121 °C for 15 min, it was allowed to cool to a temperature of 50 °C and, under sterile conditions, it was placed in Petri dishes, thereby obtaining 20 ml of culture medium waiting for gelation.

Various concentrations of each metal ion (25-800 ppm) were added. The metal salts (copper sulphate and lead nitrate) were used to prepare stock solutions (800 ppm).

Each plate was inoculated with a mycelial disk of the fungus by using a 0.5 cm diameter punch previously sterilized. Plates without metals ions were also inoculated as controls [16]. The plates were grown in a culture oven at 28 °C. The mycelia growth in each plate was observed during a whole period of the fungus development and then compared with the control plate by measuring in centimeters the growth diameter of each plate. All samples were treated in triplicate. The statistical analysis was performed by using the Categorical Multifactorial ANOVA, under a completely randomized experimental design with factorial arrangement 3x6x1, a Petri dish as an experimental unit and three repetitions. The growth diameters of each fungus at the different concentrations (PRGI) were taken as reference.

3. RESULT AND ANALYSIS

3.1. Chemical analysis and visual identification of minerals of mining waste material

As a result of the chemical characterization of the mine residues presented in Table 1, iron is determined as the major component, whereas zinc, calcium, manganese, aluminum, potassium and copper are secondary components. Regarding the presence of heavy metals, lead is found in first place, followed by cadmium and silver, this last is present in a small proportion. Moreover, the minerals from the pile showed a leachate with a paste pH slightly basic essentially due to the presence of carbonates in its composition. These mining wastes are the result of the accumulation of mineralized rocks which have been subjected to the process of alkalization with slacked lime, and later leached with sodium cyanide in order to extract the valuable minerals.

The visual identification of minerals of the mining waste suggested the presence of: pyrite (Py), Quartz (Qz), clays, mica, rock clasts as well as of oxides (Figure 2), and other supplementary material. In the sample, a strong reaction to the addition of cold HCl was observed, which shows the presence of calcium carbonate.

For this reason, the chemical-mineralogical characterization corresponds with that of minerals from a lixiviation pile, where the protective alkalinity of a pH higher than 10 is achieved by adding measured quantities of alkali; this is typical of a lixiviation process with cyanide to dissolve gold and silver.

Table 1. *Data of elements determined through ICP.*

Cu (μg/g)	Fe (μg/g)	Cr (μg/g)	Al (μg/g)	Pb (μg/g)	Si (μg/g)	Na (μg/g)
1722	183400	7.90	10930	5045	172.3	3927
Mn (μg/g)	Ca (μg/g)	Mg (μg/g)	Zn (μg/g)	P (μg/g)	Cd (μg/g)	Li (μg/g)
18370	108000	3418	35850	N/D	159.70	41.80
K (μg/g)	Mo (μg/g)	Ag (μg/g)	Sr (μg/g)	Ti (μg/g)	Ni (μg/g)	Co (μg/g)
5681	24.30	6.80	133.10	N/D	0.70	N/D

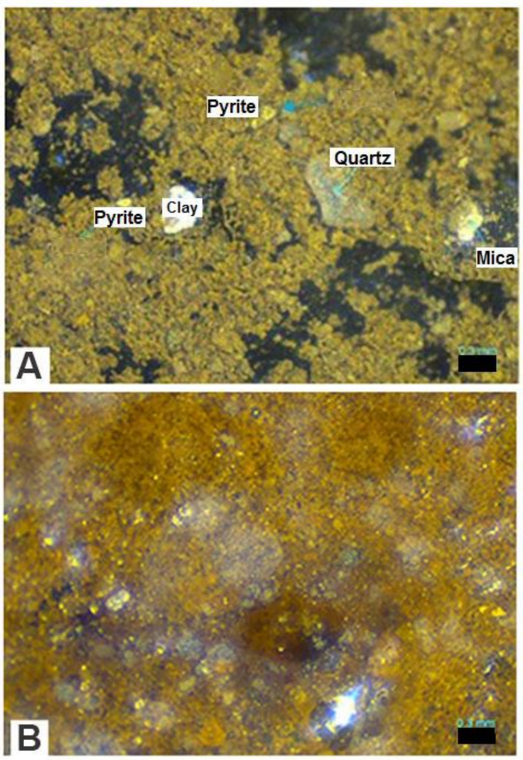

Figure 2. *(A-B). Image of mineralogy of leach pile.*

It is considered that the chemical-mineralogical characterization of the mining waste is clear, as the final objective was to show the presence of heavy metals in the mining waste, which was precisely the place from where these filamentous fungi capable of growing in those contaminated environments were isolated.

3.2. Identification of filamentous fungi

As a result of this study, strains of the genera *Fusarium* and *Penicillium* were found (see Table 2). According to Cárdenas, *et al*., 2015, several genera of fungi were isolated from rivers in México, namely *Alternaria sp*, *Penicillium sp*, *Curvularia sp*, *Aspergillus Niger*, *Aspergillus flavus*, *Cephalosporium sp*, *Fusarium sp and Cladosporium sp* [17]. The isolated fungi show different patterns of resistance to the metals analyzed: most grow in lead and zinc, few in mercury and arsenic, and the highest sensitivity found was that of cadmium. These fungi can be used for the uptake of heavy metals in solution either alone or accompanied by other biomasses as reported in the literature [17, 18]. A potential heavy metal biosorbent of the fungal type of the genus *Penicillium sp.* was isolated from the contaminated soils of the El Alacrán Mine (Colombia). The experimental results showed that the fungus had a high capacity to eliminate: Pb (II), Cd (II) and Hg (II) in an aqueous solution [19]. Holanda *et al*., (2016), reported that a sample of soil from a mine in the province of Chonnam in South Korea which was contaminated with heavy metals was an important source of biosorbents. The chemical analysis showed high contents of lead (Pb) at 357 mg/kg and cyanide (CN) at 14.6 mg/kg in the soil. The experimental results showed that *Penicillium sp*. MRF – 1 was the best lead resistant fungus among the four species of fungus tolerant to heavy metal isolated from the soil [20]. Moreover, the literature reports the isolation of a native strain identified as Klebsiella sp. 3S1 biofilm isolated from a wastewater treatment plant in the old mining district Linares-La Carolina located in Sierra Morena (España) [21].

One strain of each species was macro and microscopically identified as detailed below:

Penicillium simplicissimum A: it has double whorl brushes and lanceolate phialides with colonies of a diameter of less than 10 mm after 7 days of incubation in G25N medium. Colonies in CYA medium (with a diameter of 35-50 mm) are radially sulcate or have irregular flocculated wrinkles and dense, white-grey mycelium. Conidia production is low to moderate. These are greyish green to dull green, sometimes they look more yellow or olive. The obverse of the colony was pale yellow. Colonies in MEA usually have a diameter of 35-45 mm, and are floccose; they have a white or pale mycelium; the conidia are similar to those of CYA in color; the reverse is pale, brownish, dark brown, dark green, or most of the times pink, centrally or in sectors, and occasionally even bright red. Colonies occur in G25N of 10-18 mm in diameter with typically flat, low, from soft to floccose white or yellow mycelium, greenish-grey conidia with a pale reverse. At 37 °C, colonies are 10-30 mm in diameter, dense and hairy, with a clear exudate and brown soluble pigment, and they have a pale yellow reverse. The colonies show spherical conidia with smooth walls, Figure 3 (a) and (b).

Fusarium sp M: genera characterized by the production of small hyaline, unicellular conidia, which are born individually from solitary phyllodes. The conidia are born sequentially, but they are not connected to each other. They show variability in the structures responsible for both, forming the cells, and for asexual reproduction. The colony is white in ASG medium, but pink in PDA medium, Figure 3 (c) and (d).

Table 2. *Molecular identification of fungi isolated from a leach pile.*

Fungi	Access number GenBank	Closet Match (UNITE)	Identification (%)
A	EF634422	SH182481.07FU *Penicillium simplicissimum* (Samson *et al.*, 2011)	100
M	EF453114	SH219102.07FU *Fusarium sp*	98.71
B2	GQ221866	SH209380.07FU *Penicillium funiculosum* (Samson *et al*., 2011)	100

Penicillium funiculosum B2: colonies in CYA are approximately 250 mm in diameter; they are flat, crusted, hairy, and sulcate with dark red color; they produce soluble reddish pigment and few transparent exudates. They show whitish to reddish conidia. Colonies in MEA are approximately 300 mm in diameter, creamy in appearance and with reddish excrescences toward the center. Although the front face of the colony does not show sulci when seen from above, it presents well marked rugosities. It produces transparent to reddish exudates. Conidia are from whitish to greenish in color. Growth is almost undetectable at 37 °C; conidia do not grow in G25N or at 4 °C. They have complex double whorl conidiophores which are branched. Occasionally, they also have one-whorl conidiomers; they have lanceolate phialides, smooth stipes and rounded cones with smooth walls, Figure 3 (e) and (f).

3.3. Tolerance to heavy metals

The application of the analysis of variance (ANOVA) is shown hereunder, where the variability associated with each factor, the interaction among them, and the statistically significant terms are studied. In Table 3, the case of lead is shown, and in Table 4, the case of copper is shown.

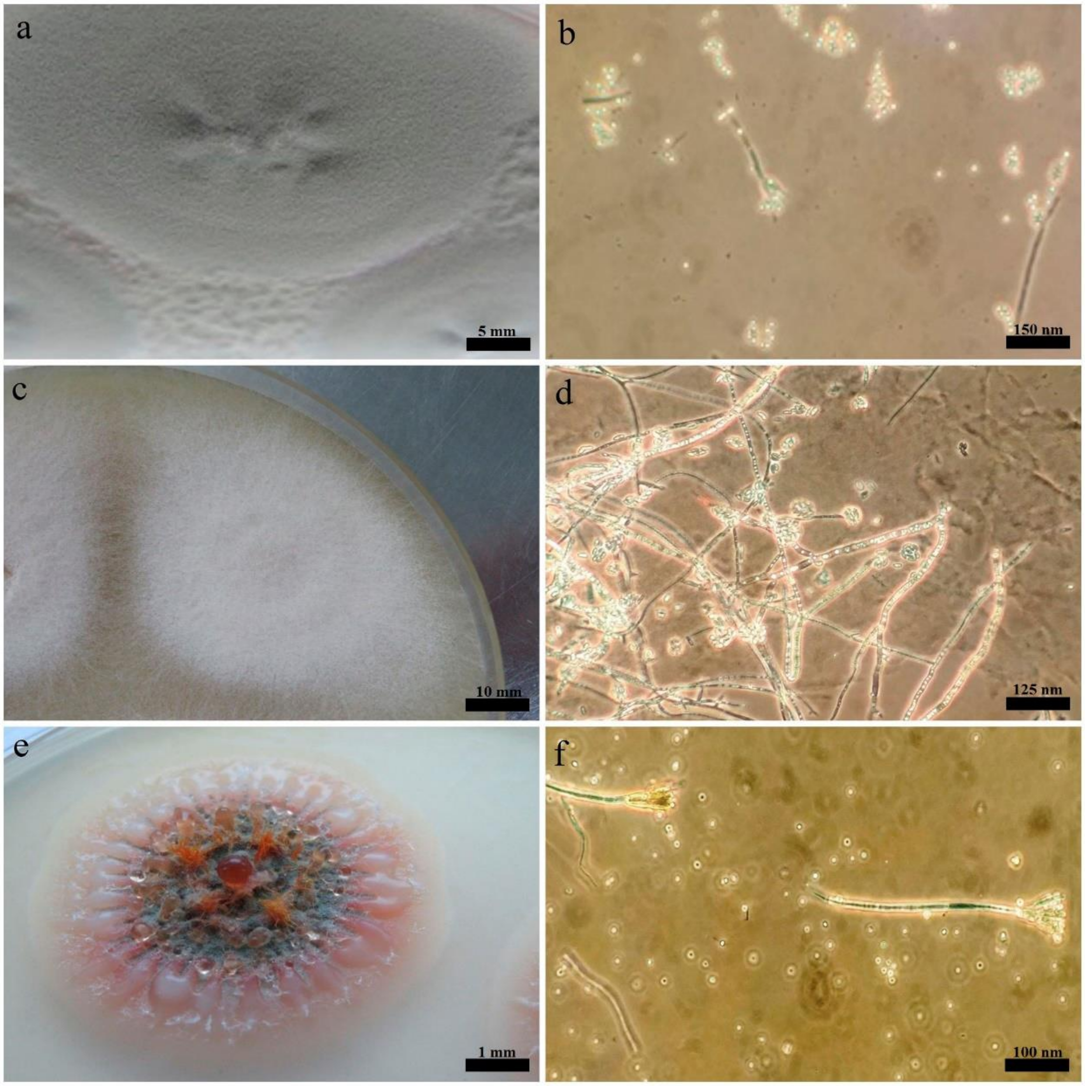

***Figure 3**. Macroscopic morphology of Penicillium simplicissimum colony in G25N medium **(a)**. Detail of spherical conidia in optical microscope with phase contrast at 40× magnification **(b)**. Macroscopic morphology of Fusarium sp. colony in ASG medium **(c)**; Detail of conidia in optical microscope with phase contrast at 40 × magnification **(d)**. Macroscopic morphology of Penicillium funiculosum colony in MEA medium **(e)**. detail of conidiophore in optical microscope with phase contrast at 40 × magnification **(f)**.*

***Table 3.** Analysis of Variance to PRGI for the metal lead – Sum of Squares Type III*

Source	Sum of squares	d.f	Mean Square	F- Value	P-Value
MAIN EFFECTS					
A:Fungus	10728.4	2	5364.18	37.81	0.0000
B:concentration of Pb	3717.4	6	619.566	4.37	0.0144
RESIDUALS	1702.39	12	141.866		
TOTAL (CORRECTED)	16148.1	20			

***Table 4.** Analysis of Variance to PRGI for the metal copper – Sum of Squares Type III*

Source	Sum of squares	d.f	Mean Square	F- Value	P-Value
MAIN EFFECTS					
A:Fungus	13750.3	2	6875.17	23.51	0.0001
B:concentration of Cu	10295.0	6	1715.83	5.87	0.0046
RESIDUALS	3508.8	12	292.4		
TOTAL (CORRECTED)	27554.1	20			

The ANOVA tables decompose the variability of PRGI in contributions due to various factors. Taking into account the fact that the Sum of Squares Type III was chosen, the contribution of each factor is measured by eliminating the effects of the other factors. The values-P proves the statistical significance of each of the factors for the metals studied, as they were lower than 0.05. Therefore, it can be concluded that these factors have a significant statistical effect over PRGI with a confidence level of 95.0%.

As can be seen in Table 5, the strains showed adequate tolerance to Cu (II) and Pb (II) at various concentrations. The Percentage of Radial Growth Inhibition (PRGI) is also shown in this table. For this purpose, a range of growth has been taken as reference, starting with the initial diameter of the inoculum of each fungus until reaching its total development, which, at most, is the extent of the complete plate; i.e. the initial proportion of inoculum was 0.5 cm and the final ratio growth extent of the fungus in the whole of the plate was 4.25 cm.

A value of PRGI = 0 % corresponds to the total tolerance of the fungus to the metal, while a PRGI value = 100 % corresponds to fungi not tolerant to metal.

It may be claimed that out of the two metals assayed at various concentrations, lead was the most highly tolerated by the three strains, even more than copper (Pb > Cu).

Table 5. *Tolerance of Fusarium sp M, Penicillium funiculosus B2 and Penicillium simplicissimum A to heavy metals and assessment of tolerance capacity expressed in PRGI.*

Metal concentration (ppm)	M-H1 *Fusarium sp*	B2-H2 *Penicillium funiculosum*	**A-H3 *Penicillium simplicissimum***
	Values of PRGI		
Pb 25	15.60	49.87	0.00
Pb 50	16.67	50.67	0.00
Pb 75	19.02	53.33	0.00
Pb 100	20.00	54.67	0.00
Pb 400	32.25	54.67	6.40
Pb 600	53.33	70.00	7.52
Pb 800	85.33	79.60	11.63
Cu 25	0.00	36.00	0.00
Cu 50	0.00	59.20	0.00
Cu 75	17.87	63.60	0.00
Cu 100	22.27	78.40	0.00
Cu 400	35.56	83.56	5.23
Cu 600	78.27	91.60	8.02
Cu 800	86.67	100.00	10.50

In particular, the PRGI values of the present study showed that the *Penicillium simplicissimum* strain was the most tolerant to both metals, exhibiting zero radial inhibition percentages for the lowest concentrations (from 25 ppm to 100 ppm) of both lead and copper. Statistically, significant tolerance differences (PRGI) exist (P=0.00) in all the strains from which the *Penicillium simplicissimum* A was the fungus showing the highest tolerance (lowest PRGI), as shown in Figures 4 and 5, compared with the other two fungi. For the case of lead at a concentration of 800 ppm, the PRGI value is 11.63% whereas for Cu at the same concentration, is 10.50%. The method currently used to discriminate between the means is the Duncan's multiple comparison procedure. The results obtained in this study with this method were that the Pb concentrations with the lowest and also homogeneous PRGI, did not give significant differences in the PRGI values, and were the lowest ones in the 25 to 400 ppm range. Those producing significant differences were those with the highest metallic concentrations, that is, from 600 to 800 ppm. Moreover, the Cu concentrations with the lowest PRGI and more homogeneous, which did not have significant differences in the PRGI values, were those within the 25 to 100 ppm range, whereas those producing differences, i.e., they had significantly high PRGI values, were those from 600 to 800 ppm. From these results it was possible to establish the following: both for copper and lead, the highest metallic concentrations, which is from 600 to 800 ppm, have significantly high values of PRGI. The statistical residue analysis showed that the wastes had a normal behavior and were independent.

The *Penicillium funiculosum* fungus shows a better performance in smaller concentrations of lead and copper up to 50 ppm with respect to higher concentrations of both metals respectively, where it shows a limited performance. Moreover, *Penicillium funiculosum* is more tolerant to higher concentrations of lead than of copper, and is not tolerant to the highest concentration of this latter metal (800 ppm).

The *Fusarium sp* fungus is tolerant to concentrations of up to 600 ppm of lead and 400 ppm of copper while it is not so at the highest concentrations (600 and 800 ppm) of both metals. This is consistent with the fact that fungi are a versatile biosorption group as they can grow under extreme conditions of pH, temperature and nutrient availability and high metal concentration [22,23]. Microorganisms have developed survival strategies for habitats contaminated with heavy metals through various detoxification mechanisms such as biosorption the purpose of which is to be used for the design of bioremediation processes. These offer the advantage of having cell wall components that exhibit excellent metal binding properties [24-26].

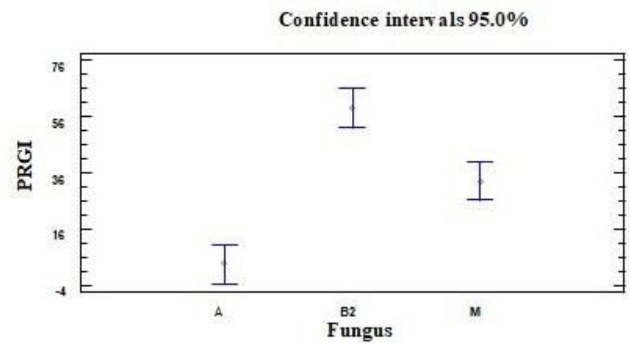

Figure 4. *PRGI graph versus the three fungi isolated for lead concentrations: M-H1, B2-H2, and A-H3.*

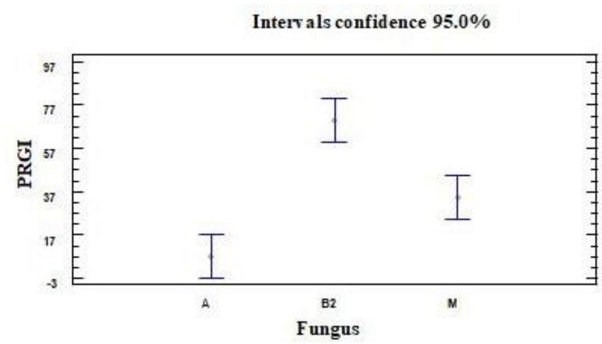

Figure 5. *PRGI graph versus the three fungi isolated for copper concentrations: M-H1, B2-H2, and A-H3*

In conclusion, taking into account the above data, it can be said that for a PRGI equal to zero or near zero it can be stated that the fungus is tolerant or highly tolerant to heavy metals, in this case lead and copper, which means that it can be used in the process of bioremediation. The type of technique used in this study allows identifying and selecting which is the most tolerant fungus.

4. CONCLUSION

Leach piles from the old Hualilán mine, from which gold and silver were mined, are characterized by their content of dissolved metals and they represent a natural source of microorganisms. Because of this, it was possible to isolate 3 strains of fungi from these mining wastes, the fungi being tolerant to high concentrations of lead and copper. Therefore, it can be concluded that one of the most recognized methods for obtaining native microorganisms is through the isolation of contaminated sites. These findings provide new candidates for a future heavy metal bioremediation process, and will singnificantly expand our knowledge about the usefulness of these microorganisms.

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