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Heavy Metals Tolerance Potential of Fungi Species Isolated from Gold Mine Tailings in Ghana

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ABSTRACT

Introduction: Heavy metal contamination has necessitated a less expensive and non-destructive clean-up technique such as mycoremediation. This study aimed to isolate, identify, and evaluate the tolerance of fungi species in different concentrations of heavy metals for their potential use in bioremediation.

Materials and Methods: Fungi were isolated by serial dilution and spread plate techniques from gold tailings and their tolerance to different concentrations of As, Cd, Cr, Cu, Pb, Zn (as potential bioremediation candidates) was evaluated. Fungal radial growths were recorded daily over a 14 days' incubation period to establish their tolerance levels using the Tolerance Index.

Results: Five isolated fungi species belonged to the genera *Aspergillus*, *Trichophyton*, *Rhizopus*, *Trametes*, and *Trichoderma*. Except for *Trichophyton rubrum*, the other fungi species were tolerant to all Cr concentrations (0 – 100ppm), but no significant difference was observed in mycelia growth compared to their controls. With high tolerance index ranging from 0.91 to 1.02, *Trichoderma viride* and *Rhizopus oryzae* were tolerant to all Cu concentrations (0 – 125ppm). High tolerance was exhibited by *Trametes versicolor* to Cd at 25 and 50 ppm, and by *Rhizopus oryzae* at 25 ppm, but no significant difference was found in mycelia growth. *Rhizopus oryzae* tolerated all As and Pb concentrations with high tolerance index ranging from 0.81 to 1.00. It also tolerated Zn at 200-600ppm concentration with a tolerance index from 0.80 to 0.91.

Conclusion: The selective nature of these fungi species for specific heavy metal tolerance indicates their potential for selective use as effective bioremediative clean up agent of heavy metals contaminated sites.

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Introduction

Increased levels of heavy metals above their background concentrations in environmental receptors through anthropogenic activities have necessitated many clean-up strategies for restoration. Presently, many physical and chemical clean-up strategies such as soil washing and flushing, encapsulation (solidification and stabilization), vitrification, electrodialysis, vapour extraction, ion exchange, and reverse osmosis are used to remediate heavy metal contaminated medium. These techniques are considered highly costly with low efficiency and are environmentally destructive [1]. As a result, an eco-friendly and less expensive remediation option that uses microorganisms isolated from contaminated sites is utilized for the restoration of contaminated environmental receptors. Accordingly, heavy metal resistant microorganisms play a vital role in the bioremediation of heavy-metal contaminated sites

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[2, 3]. Fungi are vital microorganisms in the bioremediation process due to their heavy metal tolerance and bioaccumulation capacity to remove metals from contaminated sites [4]. Thus, fungi are preferred candidates for bioremediation due to its mycelial nature and capability to accumulate heavy metals of all kinds [5]. Several metal tolerant fungi strains isolated from contaminated sites showed great tolerance to metals, which subsequently were regarded as good candidates to remediate contaminated sites [6].

In essence, compared to the conventional methods, using fungi species isolated from contaminated sites for remediation offers a safe, viable, less expensive, and more efficient way for cleaning contaminated sites. However in Ghana, people have limited knowledge with regard to the types of heavy metal tolerant fungi species in gold mine tailing dams and their subsequent tolerance to elevated concentrations of heavy metals as potential candidates for bioremediation. In this regard, the study aimed to identify fungi species in mine tailings that have the potential to be used for bioremediation.

Materials and Methods

Site description and soil sampling

Samples of tailings were obtained from a decommissioned Tailings Storage Facility (TSF 2) at Chirano Gold Mines Limited (CGML) (Figure 1). The mining firm is situated at 100 km south-west of the city of Kumasi, 15 km south-southwest of the township of Bibiani [7]. The concessional area is characterized by an annual dual rainfall pattern that occurs in March to July and September to mid-November. Various beneficent processes utilized by CGML include crushing, grinding (ball mill), gravitation, flotation, and carbon-in-leach (CIL) processing.

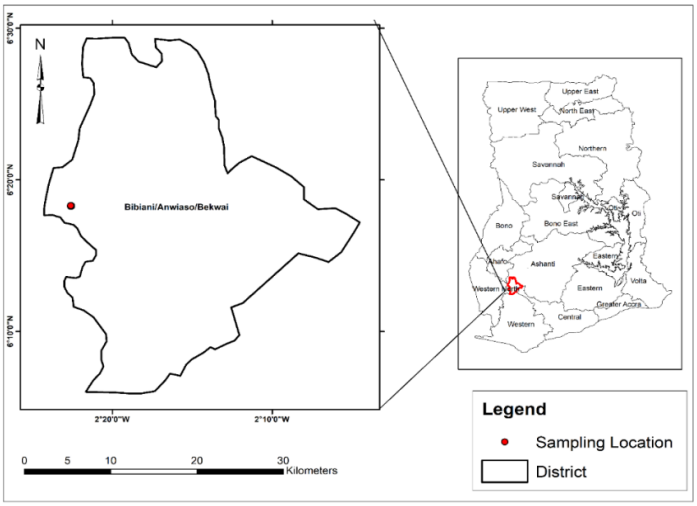

Figure 1: Geographical location of the studied area

Determination of heavy metals, pH, and electrical conductivity of mine tailings

Sample of tailings (1 g) included acid digested in a Tri-Acid Mixture (H_2SO_4: HNO_3:$HClO_4$ = 5:1:1) which was heated for 10 minutes under fumes chamber. Solution was filtered through a Whatman 1 filter paper into 50 ml volumetric flask. Later, distilled water was added to the mixture and its volume increased to 50 ml. Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), and Zinc (Zn) concentrations were determined using Atomic Absorption Spectrometer (SPECTRA AA 220 Air-acetylene Flame). Soil pH and EC were determined using glass electrode (PC 300 series - Cyberscan) in a soil-water ratio 1:2.5 [8].

Media preparation and isolation of fungi

Sabouraud Dextrose Agar (SDA) was used as the growth media. Based on manufacturer's instructions (60 g →1000 ml), 48 g of the SDA

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(4% agar) was dissolved in 800 ml of distilled water. The prepared media was then autoclaved at 121ºC for 15 minutes [9]. Bacterial growth was suppressed by adding 32 mg/l of chloramphenicol [10]. The media (20 ml) was aseptically poured into petri plates and allowed to solidify. After solidification of the media, all plates were placed at room temperature in an inverted position to avoid settling of water droplets on the media surface [11]. Isolation of fungi was performed by serial dilution (from 10^{-1} to 10^{-6}) and spread plate method. From each different serial dilution, 1 ml was spread onto a petri dish containing 20 ml of the solidified sterile SDA and incubated at 30 ± 1ºC temperature. Mycelia growth was monitored for 7 days [12]. After the incubation period, distinct colonies of fungi observed were sub-cultured for pure cultures.

Identification of fungi

Fungi were identified on the basis of their macroscopic and microscopic features [13]. The macroscopic features included colour, shape, size, and texture of colony. Microscopic features were reproductive structures, presence of sterile mycelium, septation in mycelium, shape, and structure of conidia [14]. Identities of pure cultures were established according to the literature [15]. To observe the microscopic features, fungal isolates were examined with Leica CME 1349522X light compound microscope using 0.5% methylene blue as stain under high power (×40) objective lens.

Heavy metals tolerance assessment

Preparation of heavy metal solutions and heavy metal amended media

Different concentrations of Cd, Pb, Cr, Cu, and Zn solutions were prepared from Cadmium nitrate ($Cd(NO_3)_2.4H_2O$), Lead (II) Nitrate ($PbNO_3$), Chromium (III) nitrate ($Cr(NO_3)_3.9H_2O$), Copper sulphate ($CuSO_4$), and Zinc sulphate ($ZnSO_4.7H_2O$) respectively at the Department of Chemistry-KNUST. Varying concentrations of $Cd(NO_3)_2.4H_2O$, $PbNO_3$, $Cr(NO_3)_3.9H_2O$, $CuSO_4$, and $ZnSO_4.7H_2O$ were prepared by dissolving in distilled water [16]. Arsenic concentrations were prepared by dissolving Arsenic trioxide (As_2O_3) in a beaker with 10 ml of 10% NaOH and 20 ml of concentrated H_2SO_4. Complete dissolution was obtained by gently heating on a hot plate under fumes chamber for 10 minutes. The As concentrations were 125, 250, 375, and 500 ppm. The Cd concentrations included 25, 50, 75, and 100 ppm. The Cr concentrations were 40, 60, 80, and 100 ppm. The concentrations of Cu were 50, 75, 100, and 125 ppm and the Pb concentrations included 100, 200, 300, and 400 ppm. Finally, Zn concentrations were 200, 400, 600, and 800 ppm. The pHs of solutions were maintained at 5.6 ± 0.2 by adjusting with 0.1M HCl and 0.1M NaOH [16].

Heavy metal amended media was prepared by mixing 60 ml of SDA (4%) with 30 ml of each heavy metal solutions thoroughly. The amended media was sterilized in an autoclave at a temperature of 121ºC for 15 minutes and allowed to cool at room temperature. Bacterial growth was suppressed by adding 32 mg/l of chloramphenicol [10]. The amended media (20) ml was poured into 85 mm sterile petri dishes in triplicates and allowed to solidify. To avoid contamination, set up was performed in a laminar flow [14]. Isolated pure species of filamentous fungi were then assessed for heavy metal tolerance at varied concentrations.

Inoculation of amended media with mycelia plugs

A 7 mm uniform disk of fungal species from a 7-day old actively growing pure culture on SDA was cut from each fungi species using a sterile metallic borer. They were individually inoculated on the solidified varied concentrations of each amended media aseptically in triplicates [12]. To ensure direct contact with the amended media, the test fungi was inoculated upside down at the centre of the solidified amended media. The test fungi inoculated on SDA without heavy metal served as control. All inoculated plates were incubated at a temperature of 30 ± 1ºC for 14 days. During the incubation period, fungi mycelia radial growth was monitored and recorded daily by measuring the spread of the mycelia from the centre of inoculation to the end of the longest hypha for the estimation of fungal tolerance level. Thus, each test fungal species

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tolerance to the various concentrations of heavy metals was determined by the Tolerance Index (TI) (equation 1) as prescribed by [17].

[1]

$$\textbf{T.I.} = \frac{\text{Radial growth rate of test fungal in metal medium}}{\text{Radial growth rate of the test fungal in control medium}}$$

Fungi heavy metal tolerance ratings were adopted as described [12]: 0.00–0.39 (very low tolerance), 0.40–0.59 (low tolerance), 0.60–0.79 (moderate tolerance), 0.80–0.99 (high tolerance) and 1.00–>1.00 (very high tolerance).

Higher T.I values increased the fungi tolerance to the heavy metal suggesting its potential as effective clean-up agent for bioremediation. So, fungal isolates showed greater growth after the incubation periods were tolerant to the heavy metal [18].

Statistical analysis

The obtained data were reported in mean and standard deviation using Statistical Package for Social Sciences (SPSS) version 20. Subsequently, one-way analysis of variance (ANOVA) was run to rank and compare means at 5% level of significance.

Results

Levels of heavy metals, pH and electrical conductivity of mine tailings

The tailings have an alkaline pH of 8.4 and a mean EC value of 970.92 µS/cm. Concentrations of As, Cd, Cr, Cu, Pb, and Zn were 1.44, 2.32, 1.55, 5.99, 1.81, and 3.00 mg/kg, respectively.

Diversity of fungi

Five different fungal strains were isolated from the tailings (Plate 1). Based on the macroscopic and microscopic features, the fungi isolates were identified as *Aspergillus fumigatus* (1a, 2a), *Rhizopus oryzae* (1b, 2b), *Trametes versicolor* (1c, 2c), *Trichoderma viride* (1d, 2d), and *Trichophyton rubrum* (1e, 2e).

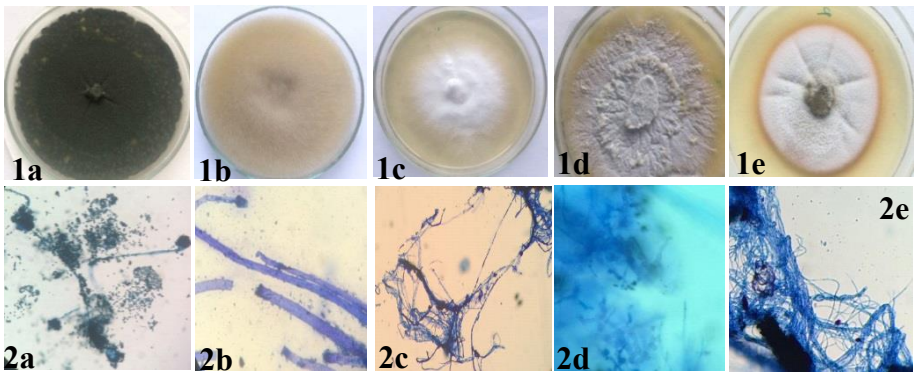

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Plate 1: Isolated colonies of fungal species from Mine Tailings (1a-1e). Photomicrograph (2a-2e) of fungal species X400. (a) *Aspergillus Fumigatus* (b) *Rhizopus oryzae* (c) *Trametes versicolor* (d) *Trichoderma viride* (e) *Trichophyton rubrum*

Fungal growth responses to various concentration of heavy metals

Mycelia radial growth of test fungal strains in various heavy metal rich media concentrations

In general mycelia growth of all fungal species was inhibited by increase of the metal concentrations. *Rhizopus oryzae* reached the maximum radial mycelia growth of 85.00 mm (Table 1) in all As concentrations (125-500 ppm) with no significant difference ($P < 0.05$) compared to the controls over the period of incubation. Mycelia growth of *Trametes versicolor*, *Trichophyton rubrum*, *Trichoderma viride*, and *Aspergillus fumigatus* were significantly inhibited in all As concentrations. Uninhibited mycelia growth in Cd concentrations was limited to 25 and 50 ppm for *Trametes versicolor* and 25 ppm for *Rhizopus oryzae*. Except for *Trichophyton rubrum*, *the* maximum mycelia growth was observed in *Trichoderma viride*, *Trametes versicolor*, *Rhizopus oryzae*, and *Aspergillus fumigatus* in all Cr concentrations.

In all Cu enriched media, maximum mycelia growth was observed for *Trichoderma viride and*

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Rhizopus oryzae, while similar growth by *Trametes versicolor* was limited to 100 ppm. Mycelia growth of *Trichophyton rubrum* and *Aspergillus fumigatus in* all Cu concentrations inhibited significantly compared to their controls. *Rhizopus oryzae* and *Trametes versicolor* obtained the maximum mycelia growth in 100 ppm, 200 ppm, and 300 ppm with regard to Pb concentrations. Similar mycelia growth was observed by *Trichophyton rubrum, Trichoderma viride*, and *Aspergillus fumigatus* in 100 ppm and by *Aspergillus fumigatus* in 200 ppm Pb concentrations. All species were significantly inhibited when exposed to 400 ppm of Pb. In Zn enriched media, maximum mycelia growth was observed for *Rhizopus oryzae* in all concentrations except for 800 ppm. Mycelia growth was significantly inhibited ($P < 0.05$) in all other fungal species relative to their controls.

Table 1: Mean radial growth (mm) of test fungal strains exposed to various concentrations of heavy metals with controls over 14 days incubation period

Fungal species	Heavy Metals As (ppm)				
	Control	**125**	**250**	**375**	**500**
Trichophyton rubrum	82.63 ± 0.13^{d}	16.88 ± 1.38^{c}	12.63 ± 0.38^{b}	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}
Trichoderma viride	85.00 ± 0.00^{e}	70.50 ± 0.50^{d}	39.75 ± 0.10^{c}	35.13 ± 0.38^{b}	25.13 ± 1.38^{a}
Trametes versicolor	85.00 ± 0.00^{d}	9.00 ± 0.00^{c}	8.75 ± 0.00^{b}	8.88 ± 0.13^{cb}	8.00 ± 0.00^{a}
Rhizopus oryzae	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}
Aspergillus fumigatus	84.67 ± 0.14^{c}	37.08 ± 0.52^{b}	31.42 ± 13.06^{ab}	25.25 ± 5.16^{ab}	18.68 ± 1.16^{a}
	Pb (ppm)				
	Control	**100**	**200**	**300**	**400**
Trichophyton rubrum	82.63 ± 0.13^{d}	83.00 ± 2.00^{d}	74.88 ± 0.13^{c}	64.25 ± 0.00^{b}	52.00 ± 0.50^{a}
Trichoderma viride	85.00 ± 0.00^{d}	85.00 ± 0.00^{d}	82.25 ± 0.00^{c}	73.63 ± 2.13^{b}	64.25 ± 0.00^{a}
Trametes versicolor	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	84.88 ± 0.13^{b}	54.75 ± 5.00^{a}
Rhizopus oryzae	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	84.25 ± 0.25^{a}
Aspergillus fumigatus	84.67 ± 0.14^{b}	78.67 ± 0.95^{b}	75.00 ± 9.78^{ab}	67.00 ± 0.90^{a}	66.92 ± 1.63^{a}
	Cr (ppm)				
	Control	**40**	**60**	**80**	**100**
Trichophyton rubrum	82.63 ± 0.13^{e}	75.50 ± 0.00^{d}	73.50 ± 0.00^{c}	69.75 ± 0.00^{b}	67.75 ± 0.50^{a}
Trichoderma viride	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}
Trametes versicolor	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}
Rhizopus oryzae	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}
Aspergillus fumigatus	84.67 ± 0.14^{a}	84.00 ± 1.00^{a}	83.80 ± 0.75^{a}	83.77 ± 1.25^{a}	83.75 ± 0.66^{a}
	Cd (ppm)				
	Control	**25**	**50**	**75**	**100**
Trichophyton rubrum	82.63 ± 0.13^{e}	60.63 ± 1.13^{d}	26.75 ± 1.75^{c}	16.38 ± 0.38^{b}	6.00 ± 0.00^{a}
Trichoderma viride	85.00 ± 0.00^{e}	70.25 ± 2.00^{d}	42.63 ± 0.13^{c}	33.75 ± 1.00^{b}	12.25 ± 0.25^{a}
Trametes versicolor	85.00 ± 0.00^{c}	85.00 ± 0.00^{c}	84.75 ± 0.23^{c}	74.75 ± 2.76^{b}	52.60 ± 0.36^{a}
Rhizopus oryzae	85.00 ± 0.00^{c}	85.00 ± 0.00^{c}	79.58 ± 1.42^{b}	76.08 ± 1.51^{a}	74.33 ± 1.46^{a}
Aspergillus fumigatus	84.67 ± 0.14^{c}	22.00 ± 0.00^{b}	17.42 ± 1.91^{ab}	17.50 ± 3.68^{ab}	14.08 ± 0.33^{a}
	Zn (ppm)				
	Control	**200**	**400**	**600**	**800**
Trichophyton rubrum	82.63 ± 0.13^{d}	40.00 ± 0.00^{c}	14.00 ± 0.25^{b}	12.88 ± 0.88^{b}	10.38 ± 0.88^{a}
Trichoderma viride	85.00 ± 0.00^{d}	39.25 ± 5.25^{c}	33.00 ± 2.00^{b}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}
Trametes versicolor	85.00 ± 0.00^{c}	67.38 ± 6.38^{b}	8.63 ± 0.38^{a}	8.00 ± 0.00^{a}	7.25 ± 0.00^{a}
Rhizopus oryzae	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	84.08 ± 0.29^{b}	83.50 ± 0.00^{b}	67.50 ± 3.00^{a}
Aspergillus fumigatus	84.67 ± 0.14^{d}	71.41 ± 7.94^{c}	41.58 ± 3.97^{b}	22.00 ± 2.00^{a}	18.00 ± 2.00^{a}
	Cu (ppm)				
	Control	**50**	**75**	**100**	**125**
Trichophyton rubrum	82.63 ± 0.13^{e}	77.25 ± 0.25^{d}	74.25 ± 1.24^{c}	70.00 ± 2.00^{b}	66.75 ± 1.00^{a}
Trichoderma viride	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}
Trametes versicolor	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	85.00 ± 0.00^{b}	83.38 ± 0.88^{a}
Rhizopus oryzae	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}	85.00± 0.00^{a}	85.00 ± 0.00^{a}	85.00 ± 0.00^{a}
Aspergillus fumigatus	84.67 ± 0.33^{c}	82.00 ± 1.00^{b}	81.00 ± 0.00^{b}	81.00 ± 0.00^{b}	70.83 ± 0.00^{a}

Mean ± SD in the same row with different superscript differs significantly ($p < 0.05$)

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Tolerance index of test fungal strains to various heavy metal concentration

The tolerance index (TI) provides a ranking that facilitates determining whether an organism is sensitive or tolerant to a particular heavy metal. Test fungal species demonstrated different levels of heavy metal tolerance (Table 2) with respect to the various metal concentrations.

Rhizopus oryzae recorded high tolerance rating in all As concentrations (125-500 ppm) with tolerance index values ranging from 0.81 to 0.93. *Trametes versicolor*, *Trichophyton rubrum*, and *Aspergillus fumigatus* showed very low tolerance with tolerance index values within the range of 0.11-0.34 in all As concentrations. *Trichoderma viride* indicated moderate tolerance at 125 ppm, but very low tolerance at 250, 375, and 500 ppm. Tolerance response to all As concentrations was in the following order: *Rhizopus oryzae* > *Trichoderma viride* > *Trametes versicolor* = *Trichophyton rubrum* = *Aspergillus fumigatus*.

In Cd enriched media (25-100 ppm), high tolerance was observed at 25 and 50 ppm for *Trametes versicolor* and at 25 ppm for *Rhizopus oryzae*. A moderate tolerance was observed at 25 ppm for *Trichoderma viride*, at 75 ppm for *Trametes versicolor*, and at 50 and 75 ppm for *Rhizopus oryzae*. Tolerance response to all Cd concentrations was in this order: *Trametes versicolor* > *Rhizopus oryzae* > *Trichoderma viride* > *Trichophyton rubrum* > *Aspergillus fumigatus*.

In Cr enriched media (40-100 ppm), very high tolerance rating to all Cr concentrations was displayed by *Aspergillus fumigatus* and *Trametes versicolor* with tolerance index value >1. *Trichophyton rubrum*, *Trichoderma viride*, and *Rhizopus oryzae* showed high tolerance in all Cr concentrations, except at 40 and 60 ppm, which were high tolerance levels for *Rhizopus oryzae*. Tolerance response to all Cr concentrations was in the following order: *Aspergillus fumigatus* = *Trametes versicolor* > *Rhizopus oryzae* > *Trichoderma viride* = *Trichophyton rubrum*.

In Cu enriched media (50-125 ppm), very high tolerance was exhibited by *Trametes versicolor* at 50, 75, and 100 ppm and by *Trichoderma viride* at 50 ppm. High tolerance levels were demonstrated by *Trichophyton rubrum* and *Rhizopus oryzae* at all concentrations, *Aspergillus fumigatus* at 50 ppm, *Trichoderma viride* at 75, 100, and 125 ppm and by *Trametes versicolor* at 125 ppm. Tolerance response to all Cu concentrations was in the following order: *Trametes versicolor* > *Trichoderma viride* > *Rhizopus oryzae* > *Trichophyton rubrum* > *Aspergillus fumigatus*.

Very high tolerance rating at 100 ppm and high tolerance at 200, 300, and 400 ppm were indicated by *Rhizopus oryzae* in Pb enriched media. The tolerance level of *Trametes versicolor* was very high at 100 ppm, high at 200 and 300 ppm, and low at 400 ppm. *Trichophyton rubrum*, *Trichoderma viride*, and *Aspergillus fumigatus* exhibited high tolerance at 100 and 200 ppm and moderate tolerance at 300 ppm. At 400 ppm, tolerance was moderate for *Aspergillus fumigatus* and low for *Trichophyton rubrum* and *Trichoderma viride*. Tolerance response to all Pb concentrations was in the following order: *Rhizopus oryzae* > *Trametes versicolor* > *Aspergillus fumigatus* > *Trichoderma viride* = *Trichophyton rubrum*.

In Zn enriched media (200-800 ppm), *Rhizopus oryzae* exhibited high tolerance levels at 200, 400, and 600 ppm and very low tolerance at 800 ppm. At 200 ppm, tolerance of *Aspergillus fumigatus* was moderate. At all other concentrations, tolerance was low to very low. Similar trend of low to very low tolerance was observed by *Trichophyton rubrum* and *Trichoderma viride*. Tolerance response to all Zn concentrations was in this order: *Rhizopus oryzae* > *Aspergillus fumigatus* > *Trametes versicolor* > *Trichoderma viride* = *Trichophyton rubrum*.

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Table 2: Tolerance index levels of test fungal strains to various heavy metals

Fungi	Tolerance index			
	As (ppm)			
	125	**250**	**375**	**500**
Trichophyton rubrum	0.21	0.16	0.11	0.11
Trichoderma viride	0.63	0.32	0.28	0.22
Trametes versicolor	0.13	0.12	0.12	0.11
Rhizopus oryzae	0.93	0.87	0.83	0.81
Aspergillus fumigatus	0.34	0.33	0.31	0.23
	Pb (ppm)			
	100	**200**	**300**	**400**
Trichophyton rubrum	0.92	0.80	0.66	0.52
Trichoderma viride	0.98	0.85	0.74	0.55
Trametes versicolor	1.00	0.92	0.81	0.42
Rhizopus oryzae	1.00	0.99	0.99	0.92
Aspergillus fumigatus	0.96	0.85	0.77	0.70
	Cr (ppm)			
	40	**60**	**80**	**100**
Trichophyton rubrum	0.93	0.91	0.86	0.84
Trichoderma viride	0.99	0.99	0.98	0.98
Trametes versicolor	1.04	1.03	1.02	1.01
Rhizopus oryzae	1.00	1.00	0.99	0.99
Aspergillus fumigatus	1.05	1.04	1.03	1.03
	Cd (ppm)			
	25	**50**	**75**	**100**
Trichophyton rubrum	0.59	0.26	0.17	0.11
Trichoderma viride	0.63	0.33	0.26	0.14
Trametes versicolor	0.87	0.80	0.60	0.38
Rhizopus oryzae	0.80	0.70	0.64	0.59
Aspergillus fumigatus	0.34	0.33	0.31	0.23
	Zn (ppm)			
	200	**400**	**600**	**800**
Trichophyton rubrum	0.32	0.19	0.18	0.16
Trichoderma viride	0.41	0.31	0.14	0.12
Trametes versicolor	0.51	0.12	0.11	0.10
Rhizopus oryzae	0.91	0.81	0.80	0.39
Aspergillus fumigatus	0.76	0.42	0.23	0.21
	Cu (ppm)			
	50	**75**	**100**	**125**
Trichophyton rubrum	0.97	0.94	0.91	0.84
Trichoderma viride	1.01	0.97	0.96	0.95
Trametes versicolor	1.02	1.01	1.00	0.90
Rhizopus oryzae	0.99	0.99	0.99	0.91
Aspergillus fumigatus	0.84	0.72	0.69	0.67

Discussion

Diversity of fungi isolates from mine tailings

Bioremediation is a technique that utilizes biological agents (microorganisms and/or plants) to reduce or eliminate the levels of hazardous substances from contaminated sites [19]. In the process of mycoremediation, the principal source of metal tolerant fungal included contaminated sites [20]. In this study, the occurrence of *Aspergillus fumigatus*, *Rhizopus oryzae*, *Trametes versicolor*, *Trichoderma viride*, and *Trichophyton rubrum* were subsequently identified in the gold mine tailings sampled from CGML. The diversity of fungi species identified was in agreement with previous studies [10, 12, 13], where the occurrence of similar heavy metal tolerant fungi strains was

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subsequently isolated from soils contaminated with multiple heavy metals or elevated levels of one or more heavy metals. Moreover, a study [12] revealed isolation of several *Trichoderma spp* from tailings sampled from Itogon, Benguet. The existence of these organisms on such sites may be ascribed to their marked adaptability to survive under extreme and various nutritional and environmental situations [22, 23, 24].

Fungal growth response and tolerance to various concentrations of heavy metals

Since fungi are natural dwellers of soil, they have greater potential for bioremediation by virtue of their aggressive growth, production of greater biomass, and extensive hyphal reach in soil [4]. In this study, *Trichophyton rubrum* and *Trichoderma viride* exhibited high tolerance to all Cr concentrations, while *Aspergillus fumigatus* and *Trametes versicolor* showed very high tolerance to all Cr concentrations. A similar study [21] confirmed Cr tolerance by *Trichoderma spp*. isolated from mine tailings sampled at Itogon, Benguet. *Rhizopus oryzae* also indicated a high tolerance to Cr. This result supported the finding of another study [13] indicating Cr tolerance by *Rhizopus spp.* isolated from heavy metal contaminated site.

Trichophyton rubrum and *Rhizopus oryzae* exhibited high tolerance to all Cu concentrations (50-125 ppm), but *Trichoderma viride and Trametes versicolor* exhibited high to very high tolerance rates to all Cu concentrations. The tolerance of *Rhizopus spp.* and *Trichoderma spp.* to high concentrations of Cu is in agreement with the findings of a similar research [12] noting that *Rhizopus spp* and *Trichoderma spp.* tolerance to Cu concentrations was up to 1000 ppm. *Rhizopus oryzae* demonstrated a high tolerance to all Pb concentrations. *Trichoderma viride* and *Trichophyton rubrum* indicated high tolerance to 100 and 200 ppm Pb concentrations with subsequent moderate tolerance to 300 ppm Pb concentration. However, increasing Pb concentration (400 ppm) resulted in significant ($P < 0.05$) reduction in mycelia growth of *Trichoderma viride* and *Trichophyton rubrum* with low levels of tolerance. *Trametes versicolor* exhibited high tolerance to Pb at 100-300 ppm. However, higher Pb concentration (400 ppm) reduced mycelia growth of *Trametes versicolor* making significantly ($P < 0.05$), which shows a low tolerance to Pb at 400 ppm. *Aspergillus fumigatus* also tolerated Pb concentration at 100-200 ppm with moderate tolerance to higher Pb concentrations (300-400 ppm). A reduction in growth rate to higher Pb concentration is a typical response of *Trichophyton rubrum*, *Trichoderma viride*, *Trametes versicolor*, and *Aspergillus fumigatus* to toxicants [25].

Trichophyton rubrum, *Trametes versicolor*, and *Aspergillus fumigatus* exhibited very little growth throughout the period of incubation with low tolerance indices value in all Arsenic concentrations (125-500 ppm). Thus, the tolerance of *Trichophyton rubrum*, *Trametes versicolor*, and *Aspergillus fumigatus* to As is considered very low affirming their sensitivity to As at 125-500 ppm. *Trichoderma viride* showed moderate tolerance to As at lower concentration (125 ppm). With As concentration at 250-500 ppm, *Trichoderma viride* exhibited very low tolerance. The slow mycelia radial growth exhibited by *Trichoderma viride* in As concentrations at 250-500 ppm affirms its sensitivity to As at 250-500 ppm. *Trichophyton rubrum*, *Trichoderma viride* in all Zn concentrations (200-800 ppm), and *Aspergillus fumigatus* in all Cd concentrations (25-100 ppm) displaced very little mycelia growth with very low tolerance to Zn and Cd, affirming their sensitivity to Zn and Cd, respectively. *Trametes versicolor* in all Zn concentrations and *Trichophyton rubrum* in all Cd concentrations also indicated little mycelia growth with low to very low tolerance to Zn and Cd affirming their sensitivity to Zn and Cd, respectively. *Trametes versicolor* exhibited high tolerance to Cd at 25-50 ppm. However, high Cd concentration (75-100 ppm) inhibited the mycelia growth of *Trametes versicolor* significantly ($P < 0.05$), *resulting* in low to very low tolerance to Cd at 75-100 ppm. *Rhizopus oryzae* also exhibited high tolerance to Cd at 25 ppm and moderate tolerance to Cd at 50-75 ppm concentrations. The

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sensitivity of *Aspergillus fumigatus*, *Trametes versicolor*, *and Trichophyton rubrum* to all As concentration, *Aspergillus fumigatus*, *and Trichophyton rubrum to* all Cd concentration, *as well as Trichoderma viride* to high Cd concentration (50-100 ppm) may be ascribed to the toxic nature of these metals [26, 27, 28]. *Rhizopus oryzae* revealed high tolerance to all as concentrations subsequently, which increased mycelia growth. This result confirms the finding of a study [12] reporting high tolerance of *Rhizopus spp.* to Arsenic up to 500 ppm.

The level of *Rhizopus oryzae* tolerance to Zn was at 200-600 ppm. At a higher concentration (800 ppm), Zn becomes extremely toxic to *Rhizopus oryzae* with significant reduction in mycelia growth rate, which subsequently exhibited very low tolerance to Zn at 800 ppm. *Aspergillus fumigatus* was also sensitive to Zn at 400-800 ppm, but moderately tolerant at 200 ppm. The low tolerance of *Trametes versicolor* to Cd at 100 ppm, *Rhizopus oryzae* to Zn at 800 ppm, and *Aspergillus fumigatus* Zn at 400 -800 ppm reflected the inhibitory growth functions of heavy metals [22].

Trichophyton rubrum and *Trichoderma viride* tolerated Cr and Cu, but were sensitive to As, Cd, Zn, and higher Pb concentrations. *Trametes versicolor* tolerated Cr, low Cd concentration, and Cu, but was sensitive to As, Zn, higher Pb, and higher Cd concentrations. *Aspergillus fumigatus* tolerated Cr and Cu, but was sensitive to As, Cd, and Zn. *Rhizopus oryzae* tolerated Cr, Cu, As, Pb, and Cd, but was sensitive to higher Zn concentration. In other words, the degree of tolerance to metals differed among different strains isolated from the tailings. Similar results were also reported in the literature [29], showing that, and various strains of fungi originating from the same metal-contaminated site did not have the same level of tolerance. Different tolerance levels may be attributed to potential variations in the tolerance mechanism utilized by the individual fungi species [30, 31]. In this regard, all confirmed *Trichoderma spp. Aspergillus spp.* were tolerance to more than one type of metal [32, 33, 34].

In principle, filamentous fungi are preferred candidates for adsorption capacities of Cu, Cr, Pb, Cd, As, and Zn in contaminated medium because they have great potential to produce large amount of biomass [35]. Similarly, large amounts of mycelia growth of *Rhizopus oryzae* were observed in all As and Pb concentrations and Zn up to 600 ppm, *Trametes versicolor* in Cd at 50 ppm, *Rhizopus oryzae* in Cd at 25 ppm, and all test fungal species to Cr (40-100 ppm) and Cu (50-125 ppm). Hence, this study confirmed that *Trichophyton rubrum*, *Trichoderma viride*, *Trametes versicolor*, *Rhizopus oryzae*, and *Aspergillus fumigatus* strains isolated from TSF 2-CGML possess inherent tolerance capacity to higher concentrations of heavy metals, indicating their suitability used for clean-up/bioremediation of heavy metal contaminated environments. Fungi that show high tolerance to toxic metals may be useful in metal recovery system [13].

All indigenous filamentous fungi isolated from the mine tailings samples, which showed tolerance to a metal exhibited high to very high tolerance to the metal. This excellent trait displaced by the isolates maybe ascribed to tolerance mechanisms utilized by fungi in the presence of a contaminated medium [12]. The suggested tolerance mechanism exhibited by fungi to heavy metals includes, extra-cellular sequestration (avoiding metal entry), or intracellular physical sequestration (reducing metal load in fungi cytosol) [36]. Extracellular sequestration was carried out via chelation, where excreted organic molecules (not part of fungi cell wall) were used to chelate metal ions. Another extracellular sequestration approach included biosorption; metals binding to cell wall due to the existence of anionic structures on fungi cell surface [8, 24, 35]. Other mechanisms included enzymatic transformation of metal ions, generation vacuoles, where metals are assembled, and immobilized in the form of polyphosphates [21, 37, 38]. The factors that enhance the capability of fungi species to tolerate heavy metal toxicity included their ability to endure extreme growth situations, secretion of effective biodegradative extracellular enzymes, and development of extensive hyphae [5].

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Conclusion

Tailings samples collected from TSF-2 were a rich source of diversified heavy metal tolerant fungi. All five test fungal strains demonstrated high tolerance for Cr. *Trichophyton rubrum*, *Trichoderma viride*, *Trametes versicolor*, and *Rhizopus oryzae* can tolerate high levels of Cu, while *Rhizopus oryzae* and *Trametes versicolor* are very good candidates for Pb tolerance. *Rhizopus oryzae* was the only species demonstrating high tolerance levels for all as concentrations of As and Zn up to 600 ppm. Although *Rhizopus oryzae* has high tolerance for Cd at 25 ppm, *Trametes versicolor* can tolerate up to 50 ppm.

Based on the growth responses and subsequent tolerance levels, the five test fungi isolates can be ranked according to their tolerance levels to heavy metals in the order; *Rhizopus oryzae* > *Trametes versicolor* > *Trichoderma viride* > *Aspergillus fumigatus* > *Trichophyton rubrum*. The exceptional mycelia growth and tolerance traits displaced by all five fungi isolates to one or more metal signify their inherent bioremediative traits. Hence, these fungi species may be potential candidates for bioremediation of heavy metal contaminated sites.

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Conflict of interests

There is no conflict of interest.

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