INVESTIGATING ARSENIC RESISTANCE IN FUNGI FROM TIN-MINING SOILS AND THE POSSIBLE INTERACTION BETWEEN ARSENIC AND TIN/ANTIMONY

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Abstract

There is an increasing interest in the study of fungi that inhabit extreme environments that can provide new biotechnological applications in treating contaminated land.

Fungi are versatile biosorbents as they can tolerate extreme levels of metal concentration, nutrient availability, pH and temperature (Gadd, 2009).

In this work, heavy metals contaminated soil was collected from Geevor Tin Mine in Penzance, Cornwall. Arsenic and antimony were found in high concentration of 18970 ± 227.0 mg/kg and 196.57 ± 1.91 mg/kg respectively in an extremely acidic soil pH of 1.13.

Acidomyces acidophilus strain shows promise in tolerating elevated levels of As (>20,000 mg/L) and Sb (>300 mg/L).

Aims/Objectives

- To provide better understanding of fungi bioremediation ability and mechanisms involve in removing arsenic.
- Isolation of arsenic resistance fungi strain from tin-mining soils using arsenic containing medium.
- Challenge the strain with the presence of antimony to establish any co-existing resistance towards arsenic and antimony.
- Determining the effectiveness of the resistant strains to remove As (with the possibility of Sb).

Methods

- **Soil sample collection**
  Soil samples were obtained from Geevor Tin Mine in Penzane, Cornwall (located at the far end of Southwest England).

Figure 1: Aerial view of the Geevor Tin-Mine site and sample collection sites (1-6) (Source: Geevor Tin Mine)

- **Investigate the soil samples heavy metal content**
  Using three-step sequential extraction and acid digestion method (Radiar & Purchase, 2012).
- **Fungi strain identification**
  Microscopic technique, DNA extraction using CTAB method, amplification and gene sequencing of ITS regions (rDNA) using PCR.

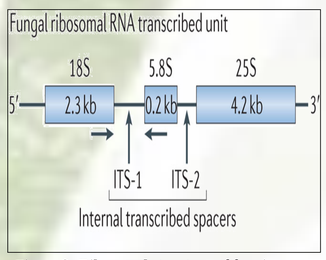


Figure 2: Ribosomal structure of fungi (source: mayoclinic.com)

- **Investigate the interaction between arsenic and antimony**
  Fungi exposed to single treatments or combinations of As and Sb using culture plates and broth.
- **Determination of fungi minimum inhibitory concentration (MIC)**
  The fungal growth were observed on minimal medium (MM) and challenged with arsenic at different concentrations to determine the MIC values.
- **Determination of metal removal efficiency**
  Metal removal efficiency (%) was determined by measuring the metal concentration remained in the growth medium using ICP-OES.

Results & Discussion

Soil analysis

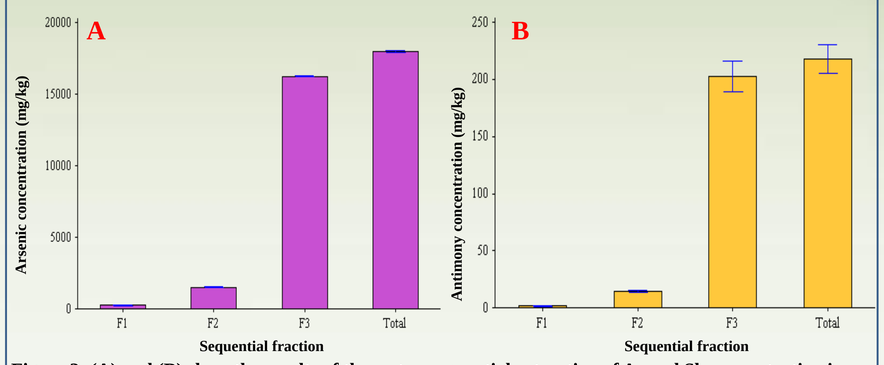
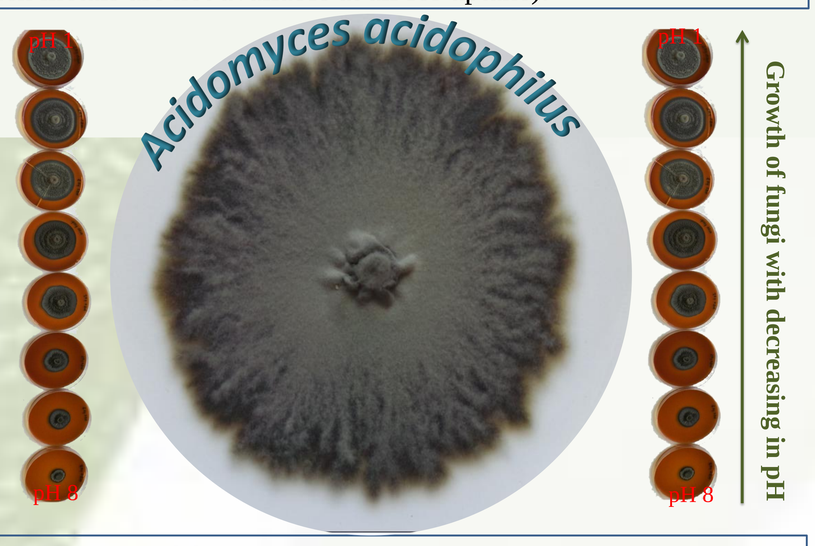


Figure 3: (A) and (B) show the results of three step sequential extraction of As and Sb concentration in site 3 soil samples.

Site 3 sample contains 18950 mg/kg of As and a three step sequential extraction was performed using this sample.

F1 fraction indicates the As and Sb bioavailability in soil samples. Fraction F2 indicates that metals are organically bound to their matrix (not readily available), while F3 is the residual fraction (which metals are not available at all for uptake).



Identification of fungal strain

- **Microscopic identification**

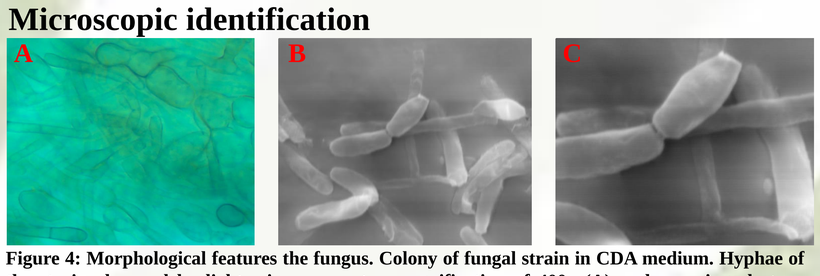


Figure 4: Morphological features the fungus. Colony of fungal strain in CDA medium. Hyphae of the strain observed by light microscope at a magnification of 400x (A) and scanning electron microscope (SEM) at a magnification of 1000x (B) and 2200x (C).

- **PCR and DNA sequencing**
  Based on the ITS and LSU regions of the rDNA, a BLAST similarity search was used to find similar sequences in the GenBank database. The ITS rDNA sequences are nearly identical to those found in ***Acidomyces acidophilus*** (Sigler & J.W. Carmich) (99.6%, AJ44237).

Minimum inhibitory concentration of fungal strain to As

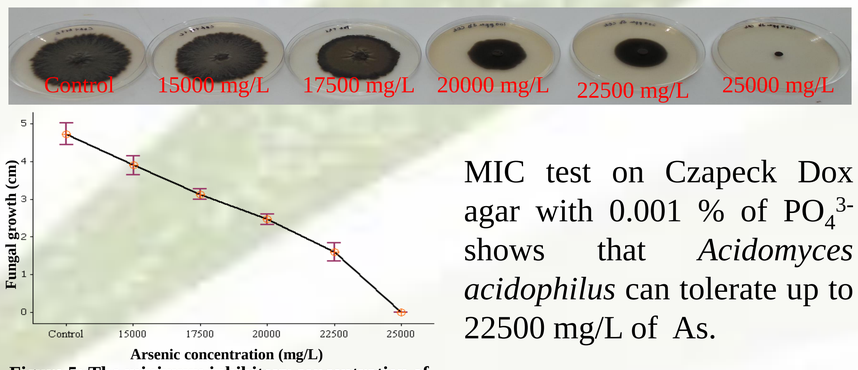


Figure 5: The minimum inhibitory concentration of As^{5+} in Acidomyces acidophilus.

MIC test on Czapeck Dox agar with 0.001 % of PO_4^{3-} shows that *Acidomyces acidophilus* can tolerate up to 22500 mg/L of As.

Biosorption of As by fungal strain

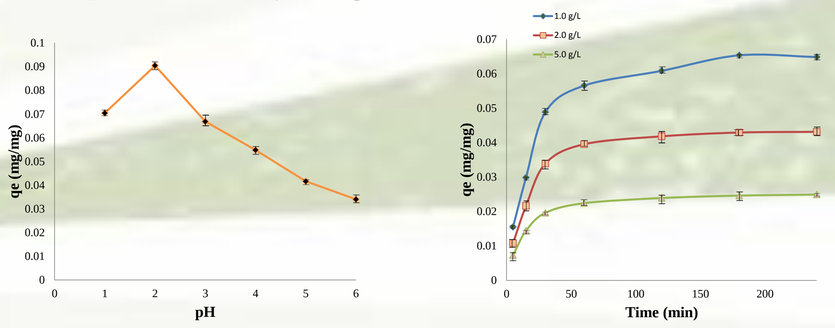


Figure 6: Effects of pH, biomass loading and contact time on As^{5+} biosorption by *Acidomyces acidophilus*.

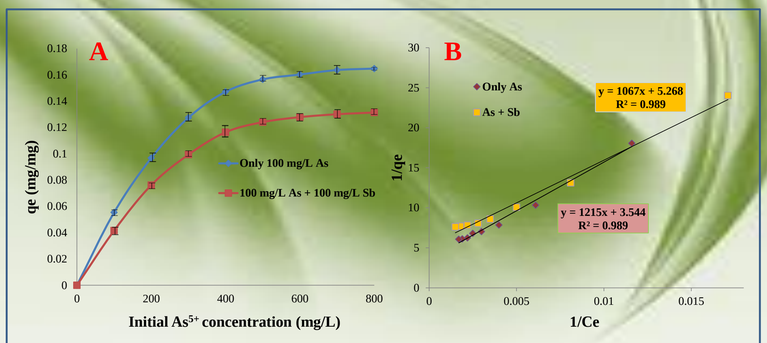


Figure 7: (A) Effects initial concentration on biosorption and (B) Langmuir plot of As^{5+} ions on Acidomyces acidophilus.

- As^{5+} adsorption by *Acidomyces acidophilus* shows the highest uptake at pH 2.
- The increase in biomass loading from 1.0 g/L to 5.0 g/L resulted in decrease of As^{5+} sorption capacity. Higher biomass loading could produce a screen effect on biosorbent surface.
- As^{5+} ions absorbed by *Acidomyces acidophilus* increased sharply within the first 45min, adsorption remained nearly constant after 180min ($p>0.05$).
- The maximum uptake was found at an initial concentration of 700 mg/L. The availability of biosorption sites were limited at concentration >500mg/L.
- The presence of Sb reduced the uptake of As^{5+} ions by *Acidomyces acidophilus*.

Adsorption isotherms

Langmuir isotherm model

$$q_e = Q \,.\, b.\, C_e \,/\, 1 + b \,.\, C_e$$

- The Langmuir model fits better than the Freundlich model on the adsorption equilibrium data in the examined concentration range of As^{5+}.
- The data from current study fitted the Langmuir isotherm model well, with regression coefficient (R^2) of 0.989 (Figure 7B). Small b values (0.027) imply strong binding of arsenic ions to *Acidomyces acidophilus*. The predicted maximum capacity of fungal strain uptake of As^{5+} was 195.3 mg/g dry biomass.

Conclusion

- *Acidomyces acidophilus* strain isolated from highly contaminated tin mining soil in Cornwall can tolerate up to 22500 mg/L of As^{5+}.
- The presence of Sb reduces the uptake of As^{5+} by *Acidomyces acidophilus*.
- Based on the Langmuir isotherm model, it predicted *Acidomyces acidophilus* has maximum uptake of As^{5+} capacity of 195.3 mg/g.

Future work

- Identify the cause of reduction in As^{5+} ions uptake by *Acidomyces acidophilus* when Sb is added during the biosorption process
- Examine the mechanisms of As uptake by fungal strain using MALDI –TOF where protein expression before and after the presence of As could provide a better understanding of As removal by fungal strain.
- Further study of other techniques involving As removal by *Acidomyces acidophilus* such as extracellular precipitation, biovolatilization, etc.

References

Gadd, G. (2009), Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment, *Journal of chemical technology and biotechnology,* vol. 84, no.1, pp. 13-28.

Radiar, A.R.B. and Purchase, D (2012) *Mathematical models to predict soil heavy metal toxicity in the 2012 Olympic site.* International Journal of Environmental Science and Technology, 9 (2). pp. 219-226.