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

W. K. Chan, D. Wildeboer, H. Garelick and D. Purchase
School of Science and Technology, Middlesex University

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 Penzance, Cornwall (located at the far end of Southwest England).
- Investigate the soil samples heavy metal content
  Using three-step sequential extraction and acd digestion method (Radar & Purchase, 2012).
- Fungi strain identification
  Microscopic technique, DNA extraction using CTAB method, amplification and gene sequencing of ITS regions (DNA) using PCR.
- 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

![Soil analysis graph](image)

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**

![Microscopic identification image](image)

**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

![MIC test graph](image)

MIC test on Czapek Dox agar with 0.001% of PO₄ shows that Acidomyces acidophilus can tolerate up to 25000 mg/L of As.

Biosorption of As by fungal strain

![Biosorption graph](image)

Figures 5: (A) and (B) show the results of three step sequential extraction of As and Sb concentration in the 3 soil samples.

**Adsorption isotherms**

Langmuir isotherm model

\[ q_e = \frac{Q_m \cdot b \cdot C_e}{1 + b \cdot C_e} \]

The Langmuir model fits better than the Freundlich model on the adsorption equilibrium data in the examined concentration range of As²⁺.

The data from current study fitted the Langmuir isotherm model well, with regression coefficient (R²) 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 As²⁺ 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 As²⁺.
- The presence of Sb reduces the uptake of As²⁺ by Acidomyces acidophilus.
- Based on the Langmuir isotherm model, it predicted Acidomyces acidophilus has maximum uptake of As²⁺ capacity of 195.3 mg/g.

Future work

- Identify the cause of reduction in As²⁺ 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