Task 3: Request for Proposals:

Mycoremediation: Bioremediation of Mining-Influenced Waters Using Fungi

Proposed, developed, and sponsored by Freeport-McMoRan Inc.

Task Summary:
Demonstrate the use of fungi in metal removal from mining-influenced water located near legacy mines.

Background
Bioremediation describes technologies that rely primarily on biological organisms (e.g., fungi, plants, microbes) to convert constituents of concern in water and soils into benign products [1]. Many bioremediation strategies are applicable to the remediation of mining-influenced waters (MIW) because of the ability of biological organisms to remove metals and other constituents of concern through various mechanisms, including biotransformation and biosorption.

Mycoremediation is one type of bioremediation that uses fungi to remove or isolate contaminants from water and soils. It is an emerging technology that is promising for removing metals in MIW [2]. Freeport-McMoRan Inc. (FMI) has discovered fungi growing in some of their bioremediation systems, though they were not purposely added to the systems. FMI invites you to explore this emerging technology to identify candidate organisms, characterize their remediation potential, and implement them in novel environmental technologies for removing metals in MIW.

Remediating Legacy Mines
There are thousands of historical mines across the United States, many of which are abandoned. These legacy mines can be very old, and many were not closed or reclaimed to current mining industry standards. Some of these mines may require remediation if MIW seepage is present.

For practical reasons (safety, access, etc.), management and remediation of seep water occurs downstream of legacy mines where the seepage is captured and treated. This is the scenario that FMI would like to explore--the use of fungi to remove metals from MIW.

Legacy-Mine Parameters and Locale
In this scenario, the MIW flows nearly year-round, at about 10 gallons per minute (gpm). Other parameters are variable across sites. Water temperatures can range from 32-80°F across sites, with temperatures typically fluctuating no more than 30° F at a given site. Some remediation sites are located at high elevations in mountainous environments while others are found in lowland or arid climates. This variability opens the doors for teams to select conditions that will make the best use of fungi to optimize metal removal.

Legacy mines are often in remote locations. Most sites do not have access to reliable power supplies or other utility infrastructure. Therefore, solutions must be self-sustaining and require little maintenance.

The treatment system will preferably be passive (e.g., operating by gravity flow without pumps or electricity), but a semi-passive system (with minimal electrical components) may be considered if the metal removal and other treatment benefits outweighs the operational costs (staff time and resources). Ideally, the system will not require power, but if the system requires power, the power source should be self-generating.

MIW Chemistry

Table 1 shows both the typical MIW chemistry and the chemistry of the synthetic solution that will be used for the bench-scale demonstration at the contest. The synthetic solution is based on the chemistry of typical MIW after pre-treatment with limestone which removes Al and Fe and increases pH. This pre-treatment helps simplify bench-scale analyses. All constituents of the synthetic solution shall be sulfate-based salts.

When preparing the solution, take care to avoid the unintended introduction of microbial contaminants (other fungi or bacteria) in the laboratory environment. Growth of such microbes is unlikely if the storage container is autoclaved, sugars are not included in the solution, and if the solution is stored at colder temperatures, such as in a refrigerator or a cold room.

Table 1. MIW and synthetic solution chemistry to be used in bench-scale testing.

MIW or solution		MIW	Synthetic Solution	Chemicals required per L of DI water
pH		3.3	~4.5-5.0	
Sulfate (mg/L)		4,500	235	
Metals (mg/L)	Al	280	-	-
	Cu	100	100	386 mg $CuSO_4 \cdot 5H_2O$
	Fe	240	-	-
	Mn	13	13	38 mg $MnSO_4 \cdot H_2O$
	Zn	40	40	199 mg $ZnSO_4 \cdot 7H_2O$

Fungi Culture and Propagation

Your team will set the parameters for maintaining the health of your fungi and the proper operation of your bench-scale system. When selecting fungi for this task, teams will consider the environmental requirements of the fungi and potential conditions at legacy mines, including the pH range of 4.5 – 5.0. To better determine the efficacy of particular fungi to remove metals, teams should focus on no more than five fungal species or fungal strains.

At the mining site, the mycoremediation system will be exposed to ambient environmental conditions that vary from site to site. Therefore, teams may choose to grow the fungi in sunlight, complete darkness, or anything in between, as the selected conditions will likely apply in at least one FMI legacy site. No restrictions will be made on survivable temperatures for the fungi, but to optimize fungi selection for FMI, the selected fungi should thrive in temperatures within a subset of 32-80°F. Teams may build a containment system to be deployed at the site that will influence lighting, temperatures, etc. during field operations, if needed.

Since the goal of the task is to determine how well fungi remove metals, the substrate on which the fungi grow should not contribute metals to the water nor act as a metal-removal mechanism.

Nutrients may be provided to establish the fungi. Nutrients may include simple sugars, oxygen (if the fungi are aerobic), removing oxygen (if the fungi are anaerobic), etc. For sustained growth of the colony, nutrients should be applicable to full-scale operations downstream of a legacy mine.

Fungal health shall be monitored via Carbon Use Efficiency measurements (CUE). This is a way to determine how much energy fungi are investing in growth vs metabolism. Higher CUE values indicate that the fungus is investing more in forming biomass and less is being “wasted” in producing CO_2 (high CO_2 production is an indication of high metabolic maintenance); values closer to zero indicate poor fungal health.

Transporting Fungus to the Contest

During transport, fungi can go into shock (slowed growth, partial die-off, or complete die-off), although a well-packed and quickly shipped system should not experience severe shock. The fungi should become fully acclimated and resume normal growth after two weeks in a new location.

As a courtesy, WERC is offering to serve as your fungal-health backup plan. You may divide your cultivated colony, then ship a portion your fungi, along with detailed care instructions, to WERC to arrive in Las Cruces, New Mexico two weeks prior to the contest. This will give the fungi two weeks to acclimate. NMSU staff in Biology, who regularly work with fungal culture, will evaluate your fungi's health when they arrive and report this to your team. They will oversee the care of your fungi until Sunday, 6 April, 2025. Note that this is only a courtesy backup to the fungi you will bring to the contest. *We cannot guarantee successful growth and acclimation of the fungi that you ship to us.*

Keep a portion of your fungi operating within your bench-scale apparatus at your home university and bring it with you to the contest, taking care to pack it appropriately. When you arrive at the contest, WERC will have your shipped fungi waiting for you in the bench-scale area. Your team can assess the health of both sets of fungi and determine which should be used in the bench-scale demonstration.

What to ship: Send the portion of your bench-scale system that includes your selected fungi and any parts of the apparatus that directly come into contact with the fungi. The goal is to ensure that the fungi can be transferred to your system (if needed) without disturbing the colony. Thus, your team may need to make one or more duplicate parts – one to send to Las Cruces along with the fungi and the second to bring with you.

Analysis: Percent Removal

Success in removing metals will be measured using percent metal removal for each of the metals listed in Table 1:

$$\%\ Removal = \left[\frac{(influent\ metal\ concentration - effluent\ metal\ concentration)}{influent\ metal\ concentration}\right]\ x\ 100$$

The goal for this task is achieving a metal-removal efficiency of 90%. Multiple passes through the apparatus are allowed.

Metal Recovery

Of interest to FMI is the recovery of metals once they are taken up by the fungi. This has the potential of being a sustainable practice that makes use of valuable resources in legacy mines and would also offset the cost of their remediation. In the technical report, propose means by which metals could be recovered from the fungi.

Contributing to the Knowledge Base

Although the team's mycoremediation system should only focus on metal removal, teams are asked to report the effect of their process on pH, oxidation-reduction potential (ORP) and Electrical Conductivity (EC). This information will not affect scoring, but it has the potential to add to the knowledge base and increase understanding about how fungi affects water quality and if it has the potential to improve water quality.

Problem Statement

Assess the efficacy of fungus as a candidate for passive or semi-passive bioremediation of MIW flowing from legacy mining sites, based on a full-scale throughput of 10 gpm.

- Propose a solution that is passive or semi-passive and minimizes waste generation.
- Select and grow fungal species that have a tolerance for metal-laden waters in a pH range of 4.5-5.0 and that can efficiently remove metal from a synthetic solution. Your team may determine other parameters, such as water temperature, aeration, and lighting conditions.
- Identify the fungal species or strains selected;
- Quantify metal-removal efficiency by the fungi, with a goal of removing a minimum of 90% of the metals;
- Suggest strategies, in the technical report, for recovering metals from the fungi.

Design Considerations

Your proposed design should provide specific details and outcomes as follows.

- Design a continuous metal-removal process on the bench scale that is scalable to a system that operates at 10 gpm.
- Identify one or more species or strains of fungi (maximum of 5) that can remove metals and thrive in a synthetic solution of chemistry indicated in Table 1.
- Establish an appropriate fungal-colony growth strategy that would be easily implemented at bench scale and at full scale at a legacy mine site.
- Determine amounts/types of nutrients and substrate necessary to establish and stimulate the growth of your selected species of fungi in the specified water chemistry.
- Ensure that the substrate does not contribute additional metals nor remove metals. Do not add pre-treatments to sequester metals. Other properties of the mine waters, such as pH, may not be altered.
- Include a Process Flow Diagram (PFD) for the selected treatment process. The PFD must include mass and energy balances (input and output streams, reactants, reaction rates, etc.). This is a major component of judging. Take extra care to ensure that all processes and waste streams are included. (See Team Manual for PFD examples).
- Determine the hydraulic retention time to treat 10 gpm. This will help determine the volume needed for the full-scale treatment system and help with costing of the system.
- Research and follow state and federal permitting requirements related to fungi treatment and discharge of treated water.
- Determine the efficacy of your strategy by reporting:
    - Metal-removal efficiency of at least 90%.
    - Viability for full-scale implementation (consider fungal colony size, growth, maintenance, residence time required for remediation of metals, etc.).
    - Mechanisms responsible for metal remediation.
    - Physicochemical parameters (pH, ORP, electrical conductivity, temperature) for both the influent and effluent and CUE for the effluent.
    - Potential feasibility for recovering metals from the fungi addressed in the technical report.
    - Waste management strategies and/or alternative uses for fungi that have absorbed the metals: What should be done with them if metals can/cannot successfully be removed?

- Present a Techno-Economic Assessment and Analysis (TEA) to construct a full-scale treatment system designed to treat up to 10 gpm of mining-impacted water.
  - Consider creating a multi-disciplinary team by inviting a business major to help draw up economic plans for full-scale implementation of your designs.
  - Capital expenses (CAPEX) typically include, but are not limited to, equipment, pipes, pumps, etc. needed to set up the system. Do not include costs of buildings and appurtenances to the treatment process.
  - Operating expenses (OPEX) should be calculated as cost to grow and maintain the fungi and dispose of any waste products. Costs should include, but not be limited to, materials needed, including consumables (chemicals, sacrificial components, etc.) In addition to other operating costs your team identifies, include these: staff labor rate ($70/hour); solids disposal costs ($50/ton).
  - Evaluate the potential of metals to be successfully recovered from the fungi and estimate the income that might be gained from metal extraction after subtracting estimated costs of the process.
  - Visualization tools: Sensitivity analyses, etc.
- Any chemicals used must be identified in the experimental safety plan (due by February 26, 2025).
- Once a successful bench-scale system is established, if desired, send a back-up of the fungal portion of the apparatus to WERC to arrive two weeks prior to the contest to allow the fungi to acclimate.

Bench-scale Demonstration

Demonstrate the use of fungi to remove metals on a bench-scale basis using synthetic mine water of chemistry outlined in Table 1. The bench-scale unit should demonstrate a continuous process that can be scaled up to a plant that treats 10 gpm of mining water.

Teams will prepare the synthetic feed solution for bench-scale testing at their home institution and WERC will provide each team with up to 5 liters of this synthetic MIW to work with during the bench-scale demonstration.

At the end of the treatment process at the contest, each team will submit for analysis four 125-mL aqueous samples: 1 sample of influent (synthetic solution) and three samples of their mycoremediated solution (effluent).

The bench-scale area is in an indoor air-conditioned area. If needed, your system may be kept outdoors—check the day/night climate conditions expected in Las Cruces in early April. It is reasonable to provide your system protection from the elements, but environmental controls beyond those that would be feasible in mining waters may not be used.

<u>Prior to the contest:</u>

If your team elects to ship a back-up fungal colony, follow the instructions on page 3. Time the shipment to arrive in Las Cruces two weeks in advance of the contest. *WERC cannot guarantee successful growth and acclimation of the fungi that you ship to us.* Ship your back-up fungal colony, instructions for care, and any related equipment to:

WERC Environmental Design Contest
NMSU College of Engineering
1025 Stewart St. Room 121
Las Cruces, NM 88003-8001

Teams will provide at the contest:

Teams will bring their fungal colonies to the contest, along with all equipment needed to run the mycoremediation project. When you arrive at the contest, your team will determine which of the duplicate fungal systems (the one you shipped or the one you brought) to use during the bench-scale demonstration.

In addition to the bench-scale demonstration, teams may bring video productions, computer simulations, tabletop displays, and scale or architectural models to assist in the presentation; these inclusions can be extremely beneficial to your presentation but shall not be substitutes for the bench-scale demonstration.

WERC will provide at the contest:

- Your team's back-up fungi and any attached equipment. This will be waiting for you at your booth when you arrive at the contest on Sunday.
- Up to 5 liters (or the amount needed by your team) of synthetic solution mixed according to Table 1.
- WERC can provide bulky difficult-to-ship items such as kiddie wading pools as secondary containment.

Requests for items needed to run the bench-scale demonstration at the contest, including the amount of synthetic solution and any specific instructions for handling the fungi at the contest location, should be specified in the ESP and submitted by February 26, 2025 (see Team Manual).

Analytical Testing Techniques

Your team will run the bench-scale demonstration under the supervision of our laboratory safety specialists. They will observe as you capture a sample of your influent as well as three samples of your effluent after the solution has passed through your bench-scale apparatus.

WERC will send the samples to NMSU labs for analytical testing of both the influent and effluent for the three items below. In the technical report, include these values from tests at your home institution's laboratory.

- ICP-OES to quantify metal-removal rates. (Method SM 4500-SO4_E using a Spec 20 instrument.)
- Temperature – These values help clarify the selected fungi's incubation preferences.
- CUE - Carbon use efficiency – to estimate fungal health - Effluent collection only.

Additional Chemical Analyses

Although the team's mycoremediation system should focus only on metal removal, in the technical report, teams are asked to report the effect of their process on the parameters listed below. The reported values will not affect scoring, but this information has the potential of contributing to the knowledge base regarding how fungi might be used to improve water quality.

- pH
- ORP - oxidation-reduction potential
- Electrical conductivity

Evaluation Criteria

Each team is advised to read "Evaluation Criteria" and "Contest Scoring" in the 2025 Team Manual for a comprehensive understanding of the contest evaluation criteria. For a copy of the Team Manual, Public Involvement Plan, and other important resources, visit the WERC website: Guidelines | werc.nmsu.edu

In addition to evaluation criteria that applies to every task, Judges will evaluate your team's response to the problem statement, with consideration of:

- The effectiveness of your bench-scale demonstration in demonstrating your team's solution and how it integrates into legacy mine remediation needs.
  i. Percent of metal removed.
  ii. Fungi viability/active-growth assessment and recommended incubation techniques, as applied to a legacy mining site.
  iii. Discussion in the technical report of the potential for metal recovery from the biomass.
  iv. Energy efficiency (e.g., solar powered, gravity fed).
- Other specific evaluation criteria that may be provided at a later date (watch the FAQs online).

Experimental Safety Plan (ESP) and Required Short Course.

See team manual for details. Due dates are listed below.

Dates, Deadlines, FAQs (*dates subject to change—watch website FAQs*)

- Today: Email us to let us know you are interested in this task. We will contact you with breaking news.
- October 15, 2024 - December 31, 2024 – Early Bird Registration discount.
- December 1, 2024 - February 20, 2025: Mandatory On-demand Course: Preparing the Experimental Safety Plan. See website and Team Manual for information.
- February 17 - 26, 2025: Experimental Safety Plan (ESP) due. Include requests for water and ancillary equipment that is not listed in the task problem statement.
- March 7, 2025: Final date to register a team.
- March 31, 2025: Technical Report due
- Weekly: Check FAQs weekly for updates:
  - Task-specific FAQs: 2025 Task FAQs
  - General FAQs: 2025 General FAQs
- All dates or task requirements are subject to change. Check FAQs for updates online.

References

[1] Deshmukh R, Khardenavis AA, Purohit HJ. Diverse Metabolic Capacities of Fungi for Bioremediation. Indian J Microbiol. 2016 Sep; 56(3):247-64. doi: 10.1007/s12088-016-0584-6.

[2] Mohamadhasani, F., Rahimi, M. Growth Response and Mycoremediation of Heavy Metals by Fungus *Pleurotus* sp. *Sci Rep* 12, 19947 (2022). Doi: 10.1038/s41598-022-24349-5.