Fungi-Wastewater Capstone Team

Northern Arizona University

Environmental W.W. Engineering

Feasibility of Fungi to Remove Heavy Metals from Mine Wastewater -Proposal

Environmental W.W. Engineering

Draft #5

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May 5th, 2020

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List of Abbreviations

Abbreviation	Description
Ca	Calcium
CENE	Civil and Environmental Engineering
Cr	Chromium
COF	City of Flagstaff
EPA	Environmental Protection Agency
ENE	Environmental Engineering
g	Grams
GU	Grading Instructor
Mg	Magnesium
mg	Milligrams
NAU	Northern Arizona University
O	Oxygen
PPE	Personal Protective Equipment
SLF	Science Lab Facility
TA	Technical Adviser
USEPA	United States Environmental Protection Agency
XRF	X-Ray Fluorescence
µg	Micrograms
SENG	Senior Engineer
ENG	Engineer
LAB	Laboratory technician

1.0 Project Understanding

The following is a description of the project. This includes the purpose of the project, background information, technical aspects, challenges, and the stakeholders of the project.

1.1 Project Purpose

A study by the EPA compared twenty-three mine accidents and found that the cheapest cleanup cost was $103,000 while the most expensive cleanup cost reached $40 million [1]. A major source of metal contamination is mine waste, often resulting from mine accidents due to the failure of containment walls, piping, or other structural failures [1]. Cleanup from these types of accidents can be costly and, therefore, alternative remediation methods are of particular interest. The purpose of this project is to test the ability of fungi to remove metals from mine waste. There is also a focus on high value metal recovery to try to incentivize fungi bioremediation.

1.2 Project Background

There is existing research regarding fungi's ability to bioremediate metals from waterways. Research from Lotliker, published in 2018, shows that fungi can remove chromium metal from water [2]. The species of fungi used was *Aspergillus sydowii* which could remove 10 µg Cr (VI) per mg biomass with an initial concentration of 300ppm Cr (IV) [2]. Electron dispersive spectroscopy was used to verify that Cr_2O_3 was present inside of the biomass, indicating the presence of active Cr (VI) removal mechanisms [2]. Another journal article by Mahmound, published in 2013, shows that fungi are capable of removing calcium and magnesium metals from water [3]. This study used *Fusarium verticillioides* and showed removal amounts of up to 1000 µg Mg (II) per g biomass and 1800 µg Ca (II) per g biomass, with initial concentrations of 1265.7 ppm and 382.4 ppm of magnesium and calcium respectively [3]. Both sorption rates could be described using either the Langmuir or Freundlich isotherms models [3]. This indicates that fungi could be used for bioremediation at waste sites that result from mining or industrial accidents.

The Figures below show *Aspergillus sydowii* and *Fusarium verticillioides*, respectively. These are the fungi species used in the research studies mentioned above [4] [5].

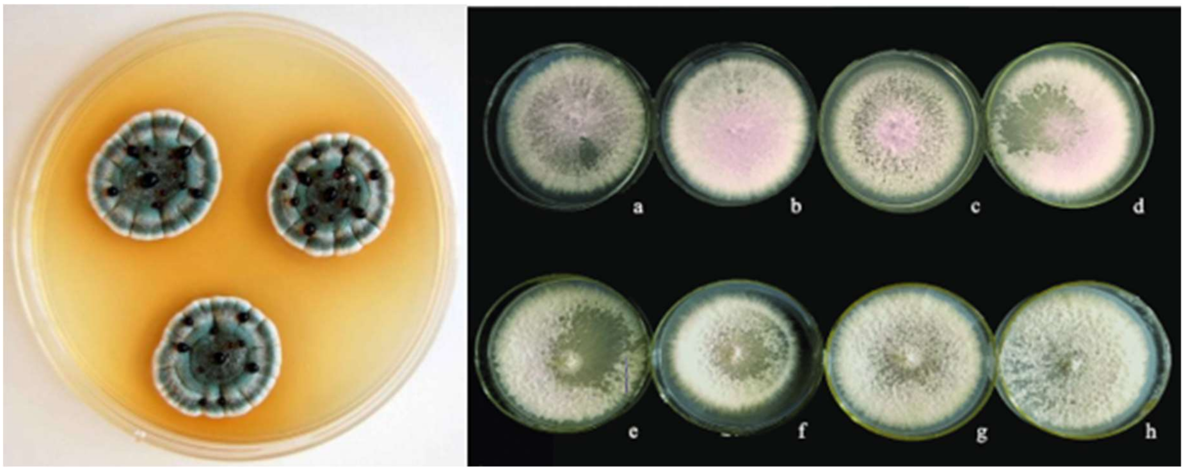

Figure 1-1: Samples of Aspergillus sydowii (left) and Fusarium verticillioides (right).

A study by Kapoor and Viraraghavan showed that *A. niger* can adsorb lead, at an initial concentration of 10 mg/L, at a higher rate when treated prior to testing [6]. This study showed that pretreatment methods can change adsorption rates by up to 700% [6]. Pretreatment done with sodium hydroxide, formaldehyde, or dimethyl sulphoxide, is capable of increasing the sorption rate, as compared to use of live biomass that was untreated [6]. Pretreatment using acetic acid, ammonium persulphate, ether and ethanol, also increased sorption rates, though the difference was less significant [6].

1.3 Technical Considerations

A technical consideration is ensuring a suitable environment to grow fungi species. The fungi species should be sheltered in a lab where the equipment needed to grow the fungi is available. A lab is also required to simulate mine waste, where the fungi species are going to be exposed to the metal contaminants. The mine waste could contain metals/metalloids such as mercury and arsenic. The lab environment will allow control over several variables that may affect the rate of biosorption. These variables may include pH, temperature, concentration of metal contaminants, mass of fungi, and the intensity and amount of light available. An XRF, or X-Ray Fluorescence spectrometer, can be used to measure the metal concentration in the samples. Adsorption isotherm experiments can be used to relate the amount of contaminant that is absorbed to the amount of biomass present. Using multiple samples with consistent concentrations of contaminants, but varying concentrations of biomass, the rate of sorption can be modeled using either the Langmuir isotherm or the Freundlich isotherm. The Langmuir isotherm is useful when the sorbent surface is homogenous, because it assumes a fixed number of sorption sites and that sorption is reversible. The Freundlich isotherm is a more empirical method of calculating sorbed concentrations because it includes sorption beyond just the surface of the sorbent.

1.4 Potential Challenges

Growing the fungi species quickly and efficiently may require highly specific conditions. The fungi species might be susceptible to changes in the environment and therefore the growing environment must be controlled. Maintaining an ideal environment for the fungi is crucial to allow the fungi to have the maximum bio-sorptive capabilities.

1.5 Stakeholders

The affected stakeholders include mining companies, as well as industries and manufacturers that focus heavily on metal recovery. Accidents, or future changes in regulations for wastewater, require cost-effective alternatives for remediating metal contamination. Large scale accidents can become so costly that it threatens to bankrupt the industry. Some of this cost could be reduced through metal recovery. The United States Environmental Protection Agency (USEPA) may also have interest in this project. When accidents occur, there is not always a party at fault, leaving any cleanup programs to be conducted by the EPA. In this circumstance, having a cost-effective and minimally invasive option for remediation would be a high priority. The public can be directly affected by this project because mine waste spills could impact the quality and availability of recreational land. Mine waste spills could also affect water resources by contamination or affect soil fertility in agricultural areas.

2.0 Research Plan

The objective of the research plan is to plan the laboratory work that is designed to prove the concept that fungi is capable of removing heavy metals from mine wastewater. The project tasks are shown in the following subsections.

2.1 Task 1: Select Fungi and Metal Contaminants

Appropriate fungi and metals for experimentation will be selected. This will require multiple subtasks including a literature review, interviews with professionals, and a decision matrix to make final selections.

2.1.1 Task 1.1: Literature Review

This task involves researching different fungi and metals to determine potential candidates for testing. The concentration of the metal that should be tested must also be researched to best simulate mine waste. Possible pretreatment methods must also be researched. At least two types of fungi and two metals will be selected as well as the media that will be used for fungi growth.

2.1.2 Task 1.2: Conduct Interview with Mycologist

The team will conduct an informational interview with Dr. Catherine Gehring, a mycologist at Northern Arizona University. The mycologist will have suggestions for choosing fungi and information about the growth of fungi. Discussions with a professional will help provide a complete view of the challenges that may occur in the following steps and how to handle them.

2.1.3 Task 1.3: Identify Selection Criteria

Criteria will be used to determine the fungi and metals that will be selected, as well the pretreatment method. Criteria will include cost, availability, toxicity, growth period (fungi), and others. All criteria used for decision making will be chosen in this step.

2.1.4 Task 1.4: Select Fungi, Metal Contaminants, Metal Concentrations

The objective of this task is to use a decision matrix to determine the fungi, metals, concentration of metals, and pretreatment techniques that will be used for testing. The criteria of selecting fungi will include the growth time, human hazard, cost, supporting research, etc. The criteria of selecting metals will include the source of the metal, effect on human health, cost, etc. The decision matrix is an important tool to aid in selecting at least two types of fungi and metals.

2.2 Task 2: Cultivate Fungi

The objective of this task is to obtain, grow, and maintain sufficient quantities of the selected fungi for analysis. This includes gaining access to laboratories, obtaining the fungi specimen, growing the fungi, and sustaining fungi health until time of testing.

2.2.1 Task 2.1: Authorize Environmental Engineering Laboratory Use

It is necessary to get access to the NAU Environmental Engineering (ENE) lab. The team members must have training certification, create a protocol binder that shows all lab work and safety requirements, and submit official requests to access the labs.

2.2.2 Task 2.2: Obtain and Grow Fungi Specimens

The fungal spawn may be provided by the NAU mycology department, City of Flagstaff (COF) Arboretum, or from certified online sources. Biomass for each fungal species will be grown independently in preparation for testing. The fungi will be grown in the biology lab at Northern Arizona University. A growth medium will be used to encourage fungi growth. Time must be considered to allow for the growth phase of the fungi life cycle. It is common for a fungi species to mature in two to four weeks in a biology lab.

2.2.2.1 Task 2.2.1: Sterilization

The growth media, an agar and nutrient mixture, must be sterilized in an autoclave before inoculation. The EnE labs contain an autoclave that may be used for sterilization.

2.2.2.2 Task 2.2.2: Inoculation and Growth

The fungi spawn will be inoculated to the selected medium in the laminar flow hood to prevent contamination. The fungi will be stored at the Science Lab Facility (SLF) building until each fungus has completely colonized the medium. The media will then be transmitted to the NAU ENE lab in preparation for testing.

2.3 Task 3: Experimental Design and Preparation

The objective of this task is to design an appropriate experiment for heavy metals removal using specific fungi species.

2.3.1 Task 3.1: Design Experimental Matrix

A heavy metal removal experiment using the fungi will be designed. The experiment matrix includes variables such as replicates, control variables, and control samples. Control variables include acidity, uptake capacity, and temperature. The repeated samples include the number of trials, while control samples represent the sample needed for quality assurance. A control sample will be made, consisting of media without a fungi specimen. An additional control sample will be used, containing the media and a deceased fungi sample that has been sterilized. The purpose of the deceased fungi sample is to compare contaminant removal rates that are a result of sorption and those of bio-uptake. The fungi will then be pretreated to increase sorption rates.

2.3.2 Task 3.2: Preparation of Experiment

Supplies, such as laboratory beakers or flasks, will be retrieved from on-campus laboratory resources or from online resources.

2.3.3 Task 3.3: Fungi Pretreatment

Pretreatment may be done with the method selected in Task 1.4. The fungi will be autoclaved to kill the fungi, oven dried, and then boiled in a solution of the chosen pretreatment chemical. The fungi will then be washed with deionized water until near pH-neutral.

2.4 Task 4: Experimentation

The objective of this task is to assess the ability of fungi in removing heavy metals from mine wastewater. Several samples will be made with varying concentrations of metals. The fungi and media must be introduced to the contaminated water, as well as the control sample, providing several points of data for a single fungi species and metal contaminant. A shaker will be used to keep the samples well-mixed for a specific amount of time.

2.5 Task 5: Data Analysis

The main objective of this task is to analyze the data related to the heavy metal concentrations, fungi maximum uptake capacity, and isothermic parameters.

2.5.1 Task 5.1: Wastewater Analysis

Metal concentration will be determined before and after implementing the fungi to find the amount of removed heavy metals. This may be done using the XRF spectrometer that is in the ENE laboratory. Flame atomic adsorption spectrometry or inductively coupled plasma mass spectrometry may also be used for identifying heavy metal concentrations.

2.5.2 Task 5.2: Fungi Analysis

Metals sorbed to the fungi will be analyzed. This may be done using the XRF spectrometer that is in the ENE laboratory. Flame atomic adsorption spectrometry, method 7000B, or inductively coupled plasma mass spectrometry, method 6020B, may also be used for identifying heavy metal concentrations.

2.5.3 Task 5.3: Create Adsorption Isotherms

The relationship between the adsorbate and the adsorbent will be determined. The adsorption isotherms will provide numerical constants that can then be used to develop mathematical relationships between contaminant concentrations and biomass concentrations. Isotherms such as Langmuir and Freundlich are expected to be used to determine the data related to the adsorption isotherms.

2.6 Task 6: Impacts

Impacts of this project will be discussed, specifically relating to the impacts on the environment, society, and the economy.

2.7 Task 7: CENE 486 Project Deliverables

Project deliverables are requested by the client to show progress throughout the project. These deliverables are required for CENE 486.

2.7.1 Task 7.1: 30% Report and Presentation

For this deliverable, the objective is to have 30% of the final report and presentation completed. The presentation will show how much progress has been made towards completing the final proposal. This includes completion of Tasks 1, 2, and 3.

2.7.2 Task 7.2: 60% Report and Presentation

The 60 % deliverable requires 60 % of the project proposal to be completed. It includes the information from the previous report deliverable as well as additional progress that has been made since the 30% report deadline. The presentation will show the progress made towards completing the final proposal. This includes completion up to Task 4.

2.7.3 Task 7.3: 90% Report and Website

The 90 % deliverable requires 90 % of the project proposal to be completed. It includes the information from the previous report deliverable as well as additional progress that has been made since the 60% report deadline. The presentation will show the progress made towards completing the final proposal. A website will be created showing the project's proposal document, presentation, and other pertinent information. This includes completion up to task 5.

2.7.4 Task 7.4 Final Report and Presentation

This task is the final report deliverable and the final presentation.

2.7.5 Task 7.5 Website

The website will be finalized, allowing the project information to be publicly accessible.

2.8 Task 8: Project Management

The project engineers are tasked with scheduling and coordinating meetings with the team, client, technical adviser (TA), and any other person(s) necessary for the project. Time in the laboratories must also be coordinated by the project engineers. Other tasks include submitting any deliverables to the client and technical adviser and preparing any other necessary activities for the project.

2.8.1 Task 8.1: Resource Management

Several resources must be managed for the project. Personnel time and funds for materials will be logged and carefully managed. Lab materials must be logged, specifically the fungi specimens and water samples which could be considered toxic substances. The resources will be recorded to ensure responsible handling of toxic materials and to ensure that the project stays under the specified budget.

2.8.2 Task 8.2: Record Meetings

Regular meetings are required with the client, technical adviser, grading instructor, and the team members. The technical adviser and client are valuable resources for technical information and will be contacted as needed. The grading instructor is a valuable resource for information on deliverables and will be contacted weekly. Team meetings will be planned weekly. The objectives of team meetings are determined prior to the actual meeting. All meetings will have an agenda, minutes documented, and recorded in a memo binder. This keeps the pace of the project moving and ensures that all team members are aware of the status of the project and the upcoming tasks.

2.8.3 Task 8.3: Project Schedule Management

The project schedule will be managed by the project engineers. The schedule outlines the times that tasks are to be completed. Changes to the schedule will only occur if necessary.

2.9 Exclusions

Field testing is excluded from this project. Although using fungi to remove metals from contaminated waterways may be an application of this research, it is beyond the scope of this project.

3.0 Schedule

The project schedule is shown in Appendix 1. The total duration of the project is 184 day, beginning on April 4th, 2020, and finishing on December 11th, 2020. There are several major tasks that require a significant amount of time to complete. The first major task is Task 2.1: Authorizing Environmental Engineering Lab Use. This step requires training, submitting a safety binder, and awaiting approval for lab use, and could take several weeks to complete. The next major task is Task 2.2: Inoculation and Growth. For this task, several weeks must be allotted for the fungi to grow and mature. The last major task is Task 6.3: 90% Report and Website. This task will take a significant amount of time because the deliverables will be considered the final deliverables, ensuring that all requirements are met and are of professional quality. Other deliverables include the 30% report and presentation, the 60% report and presentation, the final report and presentation, and the final website.

The critical path is the shortest amount of time that is possible to complete the project. The critical path consists of the following tasks: Task 1.2: Conduct Interview with Mycologist, Task 2.1: Authorize Environmental Engineering Laboratory Use, Task 2.2.1: Sterilization, Task 2.2.2: Inoculation and Growth, Task 3.3: Fungi Pretreatment, Task 4: Perform Experiment, Task 5.1: Wastewater Analysis in Wastewater Samples, Task 5.2: Fungi Analysis, Task 5.3: Create Adsorption Isotherms, Task 6.3: 90% Report, Presentation, and Website, and Task 6.4: Final Report and Presentation. The critical path is identified by predecessors. For example, interviewing a mycologist is a predecessor to design the experimental matrix, which is then a predecessor to performing the experiment. Authorization of laboratory use is a predecessor to sterilization fungi growth, both of which are then predecessors to running the experiment. The experiment must then be completed to begin data analysis and thus the final deliverables.

To maintain the timing of the project, the critical path must be the highest priority. The team must ensure that a task in the critical path is always the focus, while other non-critical tasks can be performed simultaneously. This will keep the project moving and on schedule.

4.0 Staffing Plan and Cost of Engineering Services

Table 4-1 shown below, contains the number of days the individual team members spend on each task from the scope of the project. There are three positions in this project: SENG (senior engineer), ENG (engineer), and LAB (laboratory technician). The senior engineer (SENG) is oversees the project and will be consulted when problems arise. The responsibilities of the Engineer (ENG) include planning and data review of the current task. The responsibilities of the laboratory technician (LAB) include lab work, lab procedures, and safety procedures. Table 4-1 shows the total number of hours that each position is expected to work, as well as the total person-hours of the project. The total hours of lab were calculated to be 598 hours.

Table 4- 1: Staffing Table

Staffing Table				
Tasks	**SENG (hours)**	**ENG (hours)**	**LAB (hours)**	**Total hours**
Task 1: Select Fungi and Metal Contaminants	11	14	5	30
Task 1.1: Literature Review	5	5	5	15
Task 1.2: Conduct Interview with Mycologist	1	1		2
Task 1.3: Identify Selection Critieria	3	5		8
Task 1.4: Select Fungi, Metal Contaminants, and Metal Concentrations	2	3		5
Task 2: Cultivate Fungi	25	30	65	120
Task 2.1: Authorize Environmental Engineering Laboratory Use	10	10	30	50
Task 2.2: Obtain and Grow Fungi Specimens	15	20	35	70
Task 2.2.1: Sterilization	2	3	5	10
Task 2.2.2: Inoculation and Growth	6	8	30	44
Task 3: Experimental Design and Preparation	9	7	4	20
Task 3.1: Design Experimental Matrix	5	3		8
Task 3.2: Preparation of Experiment	3	3	3	9
Task 3.3: Fungi Pretreatment	1	1	1	3
Task 4: Experimentation	5	5	10	20
Task 5: Data Analysis	31	23	33	87
Task 5.1: Wastewater Analysis	6	7	8	21
Task 5.2: Fungi Analysis	10	8	15	33
Task 5.3: Create Adsorption Isotherms	15	8	10	33
Task 6: Impacts	10	10	10	30
Task 7: CENE 486 Project Deliverables	65	61	54	180
Task 7.1: 30% Report and Presentation	8	6	5	19
Task 7.2: 60% Report and Presentation	10	10	8	28
Task 7.3: 90% Report and Website	24	24	20	68
Task 7.4 Final Report and Presentation	15	15	15	45
Task 7.5 Website	8	6	6	20
Task 8: Project Management	37	37	37	111
Task 8.1: Resource Management	3	3	3	9
Task 8.2: Record Meetings	30	30	30	90
Task 8.3: Project Schedule Management	4	4	4	12
Sum of Hours per Position	**193**	**187**	**218**	**598**

The cost of engineering services is shown in the Table 4-2. The cost of the personnel is listed, as well as the cost of supplies, and subcontracts. Supplies include rented time in laboratories, at a rate of $100 per day, as well as the cost of fungal spawn, estimated to be $25 per item. It was estimated that 4 samples will be needed. Supplies also include Personal Protective Equipment (PPE). After experimentation, samples will be sent to a lab for analytical analysis. This cost is listed under subcontracts and is estimated to be $25 per sample. The total cost of the project was estimated to be $78940.

Table 4- 2: Cost of Engineering Services

Cost of Engineering Services				
1.0 Personnel	Classification	Hours	Rate, $/hr	Cost ($)
	SENG	193	200	38600
	ENG	187	100	18700
	LAB	218	55	11990
	Total personnel cost			**69290**
2.0 Supplies	2.1 NAU lab time	20 days	$100/day	2000
	2.2 Fungal Spawn	4	$25/Item	100
	2.3 PPE			50
3.0 Subcontract	Analytical, 50 samples		$25/sample	7500
4.0 TOTAL				**78940**

5.0 References

[1] United States Environmental Protection Agency, Costs of Remediation at Mine Sites, Washington, DC: United States Environmental Protection Agency, 1997.

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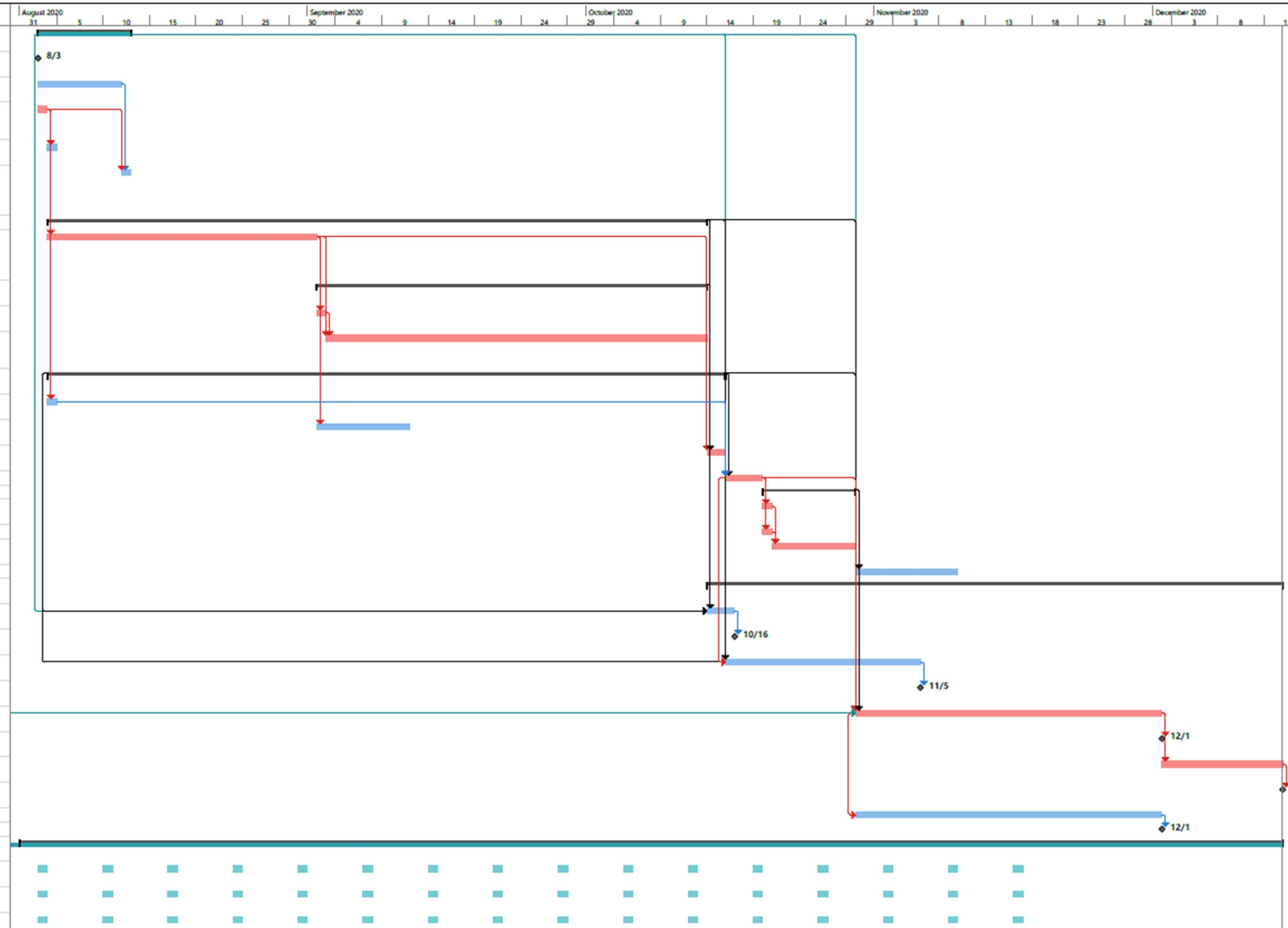
[4] "Fungi and Lichens of Great Britain and Ireland: Aspergillus sydowii," [Online]. Available: http://fungi.myspecies.info/taxonomy/term/4992/media. [Accessed 17 February 2020].

[5] X. Gai, H. Dong, S. Wang, B. Liu, Z. Zhang, X. Li and Z. Gau, "Infection cycle of maize stalk rot and ear rot caused by Fusarium verticillioides," *PLOS ONE,* vol. 13, no. 7, 2018.

[6] A. Kapoor and T. Viraraghavan, "Biosorption of Heavy Metals on Aspergillus Niger: Effect of Pretreatment," *Bioresource Technology,* vol. 63, pp. 109-113, 1998.

Appendix A-Schedule

ID	Task Name	Duration	Start	Finish
1	**Task 1: Select Fungi and Metal**	**8 days**	**Mon 8/3/20**	**Wed 8/12/20**
2	Milestone1 : Start Project	0 days	Mon 8/3/20	Mon 8/3/20
3	Task 1.1: Literature Review	7 days	Mon 8/3/20	Tue 8/11/20
4	Task 1.2: Conduct Interview with Mycologist	1 day	Mon 8/3/20	Mon 8/3/20
5	Task 1.3: Identify Selection Criteria	1 day	Tue 8/4/20	Tue 8/4/20
6	Task 1.4: Select Fungi, Metal Contaminants, and Metal	1 day	Wed 8/12/20	Wed 8/12/20
7	**Task 2: Cultivate Fung**	**50 days**	**Tue 8/4/20**	**Tue 10/13/20**
8	Task 2.1: Authorize Environmental Engineering Laboratory Use	21 days	Tue 8/4/20	Tue 9/1/20
9	**Task 2.2: Obtain and Grow Fungi**	**29 days**	**Wed 9/2/20**	**Tue 10/13/20**
10	Task 2.2.1: Sterilization	1 day	Wed 9/2/20	Wed 9/2/20
11	Task 2.2.2: Inoculation and Growth	28 days	Thu 9/3/20	Tue 10/13/20
12	**Task 3: Experimental Design and**	**52 days**	**Tue 8/4/20**	**Thu 10/15/20**
13	Task 3.1: Design Experimental	1 day	Tue 8/4/20	Tue 8/4/20
14	Task 3.2: Preparation of	7 days	Wed 9/2/20	Fri 9/11/20
15	Task 3.3: Fungi Pretreatment	2 days	Wed 10/14/20	Thu 10/15/20
16	Task 4: Experimentatic	2 days	Fri 10/16/20	Mon 10/19/20
17	**Task 5: Data Analysis**	**8 days**	**Tue 10/20/20**	**Thu 10/29/20**
18	Task 5.1: Wastewater	1 day	Tue 10/20/20	Tue 10/20/20
19	Task 5.2: Fungi Anal	1 day	Tue 10/20/20	Tue 10/20/20
20	Task 5.3: Create Adsorption	7 days	Wed 10/21/20	Thu 10/29/20
21	Task 6: Impacts	7 days	Fri 10/30/20	Mon 11/9/20
22	**Task 7: CENE 486 Project Deliverables**	**41 days**	**Wed 10/14/20**	**Mon 12/14/20**
23	Task 7.1: 30% Report and	3 days	Wed 10/14/20	Fri 10/16/20
24	Milestone 2: 30% Deliverable	0 days	Fri 10/16/20	Fri 10/16/20
25	Task 7.2: 60% Report and	15 days	Fri 10/16/20	Thu 11/5/20
26	Milestone 3: 60% Deliverable	0 days	Thu 11/5/20	Thu 11/5/20
27	Task 7.3: 90% Report and	20 days	Fri 10/30/20	Tue 12/1/20
28	Milestone 4: 90% Deliverables	0 days	Tue 12/1/20	Tue 12/1/20
29	Task 7.4: Final Report and	9 days	Wed 12/2/20	Mon 12/14/20
30	Milestone 5: Final Deliverable	0 days	Mon 12/14/20	Mon 12/14/20
31	Task 7.5: Website	20 days	Fri 10/30/20	Tue 12/1/20
32	Milestone 6: Websit	0 days	Tue 12/1/20	Tue 12/1/20
33	**Task 8: Project Management**	**93 days**	**Sat 8/1/20**	**Mon 12/14/20**
34	**Task 8.1: Resource Management**	**93 days**	**Sat 8/1/20**	**Mon 12/14/20**
68	**Task 8.2: Record Meetings**	**93 days**	**Sat 8/1/20**	**Mon 12/14/20**
102	**Task 8.3: Project Schedule**	**93 days**	**Sat 8/1/20**	**Mon 12/14/20**



Project: Schedule
Date: Tue 3/31/20

Task, Split, Milestone, Summary, Project Summary, Inactive Task, Inactive Milestone, Inactive Summary, Manual Task, Duration-only, Manual Summary Rollup, Manual Summary, Start-only, Finish-only, External Tasks, External Milestone, Deadline, Critical, Critical Split, Progress, Manual Progress