Feasibility of Fungi to Remove Heavy Metals from Mine Wastewater
- Final Report-

CENE 486C: Engineering Design

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November 24, 2020

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Acknowledgements

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
CSTR	Continuous Stirred Tank Reactor

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1.0 Project Introduction

The Feasibility of Fungi to Remove Heavy Metals from Mine Wastewater research project was designed to address novel treatment methods for high metal concentration mine wastewater. A major source of metal contamination in waterways is often related to mining accidents from the failure of containment walls, piping or other structural failures. The cost of cleaning up from these types of accidents can be high, so other remedies are of particular concern. An EPA study compared 23 mine accidents in which cleanup costs ranged from \$103,000 to \$40 million [1]. The goal of this project is to test the capability of mushrooms to remove lead and copper metals from mine wastewater.

There is existing research regarding fungi's ability to bioremediate metals from water. Research from Lotliker, published in 2018, shows that *Aspergillus sydowii* could remove 10 μg Cr (VI) per mg biomass with an initial concentration of 300ppm Cr (IV). 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 Mahmoud, published in 2013, shows that *Fusarium verticillioides* removed 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]. This indicates that fungi could be used for bioremediation at waste sites that result from mining or industrial accidents. Another study, completed by an Environmental Engineering Capstone team, found that the fungi species *Pleurotus ostreatus* had a 75% removal of E. coli through adsorption [4]. This current study is an expansion of previous capstone team's work into fungi as a biosorbent.

2.0 Selection of Fungi and Contaminants

2.1 Fungi Selection
The two fungi species that were considered were *Aspergillus niger* and *Agaricus bisporus*. The team had previously selected *A. niger* because of the extensive research using this species as a sorbent for metals in previous research. However, acquiring this species included growing the fungus, a time-consuming process that required training and access to Dr. Katherine Gehring's lab. Difficulties associated with COVID-19 concerning access to this lab necessitated a change to *A. bisporus,* shown in Figure 2.1, which is readily available in local stores.

As can be seen in the decision matrix in Table 2.1, all other fungi species considered were unavailable due to the lack of lab access.

Figure 2.1: Agaricus bisporus

Table 2. 1: Fungi Decision Matrix

Fungi Decision Matrix					
Fungi Species	Cost	Availability	Toxicity	Supporting Research	Growth Time
Aspergillus sydowii	Low	Not Available	Low	Biodegradation of caffeine by cells of Aspergillus sydowii, Aspergillus niger and optimization for caffeine degradation	Short
Fusarium verticillioides	Low	Not Available	High	Antifungal activity and inhibition of fumonisin production by Rosmarinus officinalis L. essential oil in Fusarium verticillioides (Sacc.) Nirenberg	Short
Aspergillus niger	Low	Not Available	Low	Biodegradation of caffeine by cells of Aspergillus sydowii, Aspergillus niger and optimization for caffeine degradation	Short
Aspergillus terreus	Low	Not Available	High	Enhancement of ligninolytic enzymes production and decolourising activity in Leptosphaerulina sp. by co–cultivation with Trichoderma viride and Aspergillus terreus	Short
Agaricus bisporus	Low	Available	Low	High potential to adsorb lead	None

2.2 Contaminant Selection

Several metal contaminants that are common in mine wastewater were evaluated: lead, chromium, cadmium, arsenic, zinc, and copper. A decision matrix was created to determine the best metals for the experiment. The factors that were considered were cost, availability, toxicity, existing supporting research, and typical concentration in mine waste. Lead and copper were available in the NAU Environmental Engineering Laboratory as salts and concentrates. Additionally, both elements are relatively inexpensive, while the other metals would need to be purchased. Lead is a common contaminant and due to its ubiquitous nature and significant health effects, it was selected for experimentation. Copper was selected based on its availability as well as its presence in contaminated wastewater. Upon initial testing of mushrooms and trial samples, copper was ultimately eliminated because of the existing copper concentration in the mushroom biomass.

3.0 Experimentation Preparation

3.1 Lab Binder

A lab binder was prepared to gain access to the Environmental Engineering laboratory. The binder clearly defines the objectives of the experiment, the activities that will take place in the lab, types of samples and waste being created, an emergency response plan and chemical information for all chemicals being used. Additionally, each team member completed training on Chemical Hygiene and Biosafety and Biosecurity, for which the certificates of completion are included in the lab binder. The binder is a necessary tool that ensures safety of the team during the experiment as well as information for any lab official who checks in on the work being done.

3.2 XRF Detection Limit Studies and Calibration Curve

Prior to selection of lead and copper stock solution concentrations for the sorption experiment, it was necessary to confirm the detection limits of the XRF. This device is primarily designed for use on solid material such as soils, yet in this experiment, liquid solutions were analyzed. The detection limit was determined by following the "XRF Detection Limit Procedure" which can be found in Appendix A-1. The lead and copper

stock solutions (synthetic mine wastewater) were made to have an initial concentration of 1000 ppm and then diluted in half several times. At every dilution, each known concentration was tested with the XRF by filling a sample container and placing it within the XRF apparatus. When both metals were detected by the instrument, the solution was diluted by half again and retested. This process was repeated until the concentration fell below 20 ppm, at which point the XRF was unable to read the liquid sample and reported a non-detect. This limit was deemed acceptable as evidence for the use of the XFR on liquid samples because it was well below the adsorption range needed for the actual experiment. Tables 3.1 and 3.2 display the results of the detection limit study for lead and copper, respectively.

Table 3. 1: XRF Detection Limit Test for Lead

XRF Detection Limit for Lead			
Known Concentration	**XRF Reading**		**Average Reading**
(ppm)	(ppm)		(ppm)
1000	867	--	--	867.0
500	416	426	419	420.3
250	204	212	206	207.3
125	75	74	81	76.7
62.5	26	25	25	25.3

Table 3. 2: XRF Detection Limit Test for Copper

XRF Detection Limit for Copper			
Known Concentration	**XRF Reading**		**Average Reading**
(ppm)	(ppm)		(ppm)
1000	872	858	861	863.7
500	652	647	648	649.0
250	314	308	309	310.3
125	113	109	109	110.3
62.5	23	19	23	21.7

For each pretreatment and concentration combination for the lead stock solutions, a calibration curve was developed, shown in Figure 3.1. This curve was used to correct XRF readings to actual values given that the XRF consistently reported lead concentrations lower than the actual values.
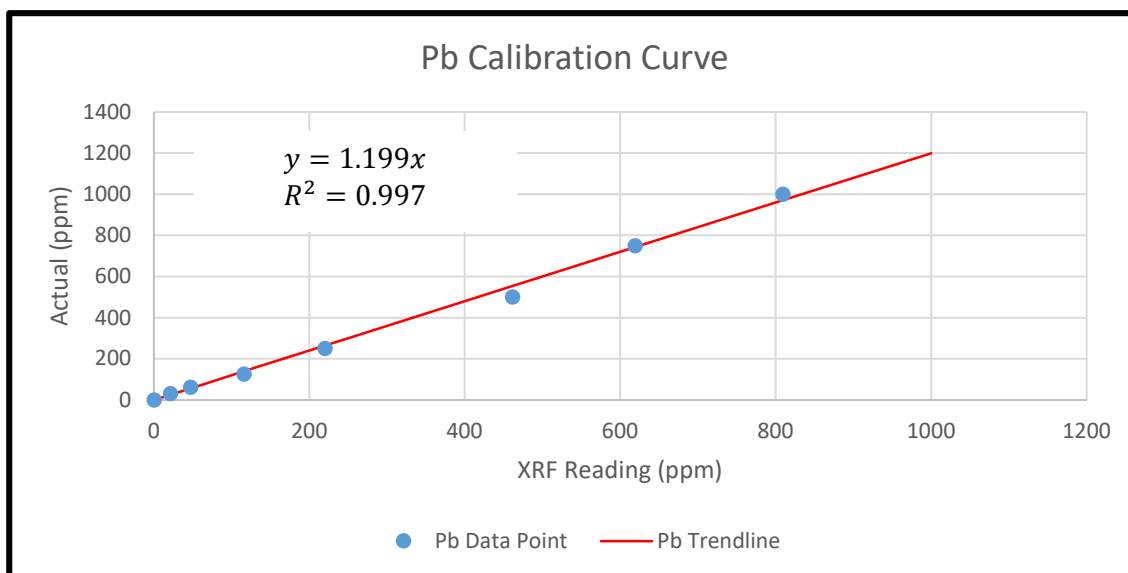
Figure 3. 1: Pb Calibration Curve

After determining the XRF detection limit, the concentration of the Pb and Cu stock solutions were chosen accordingly so that there would be detection even after sorption took place. Several studies showed that typical mine wastewater samples had pH values within the range of 4-6. Additionally, in other adsorption studies, a lower pH in the same range is also ideal for increased sorption efficiency. Therefore, the pH for each contaminant solution was tested in order to verify that the solution was within the acceptable pH range, summarized in Table 3.3. The lead stock solution is made from a highly concentrated lead acetate solution and the copper stock solution is made from a copper chloride salt. Both solutions exhibited a naturally more acidic pH after complete mixing and therefore hydrochloric acid (HCl) was not needed, as was originally expected, to lower the pH.

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Table 3. 3: Contaminant Solutions used for Adsorption Experiment

Solution	Concentration (ppm)	pH
Pb	1000	5.30
Cu	1000	4.35

4.0 Results and Discussion

The adsorption experiments conducted for this project were intended to test the adsorption capabilities of pretreated mushroom biomass in lead contaminated synthetic wastewater. This was conducted by using a consistent lead stock solution and varying the amount of biomass in each sample vial. Each adsorption experiment is outlined in Table 4.1. Ten different mushroom masses were selected, ranging from 100 mg to1000 mg. Each mushroom sample was placed in a 60 mL vial and filled with 50 mL of a 1000 ppm Pb stock solution. Then each vial was placed on a shaker table for 24 hours. The experimental procedure that was developed for this experiment can be found in Appendix A-3.

Table 4. 1: Summary of Experiment

Summary of Experiment			
Experiment	Pretreatment Method	Pb Stock Solution (ppm)	Sample Analysis Type[1]
1	Original	1000	Liquid
2	Modified	1000	Liquid
3	None	1000	Liquid
4	Modified	400	Liquid
[1]*Liquid and mushroom samples were tested with the XRF. However, the number of mushrooms used in each trial was not enough to fill the XRF sample cup, therefore, the Pb sorption was determined by a mass balance, i.e. $C_o - C_f$. The XRF readings for each sample were corrected using the calibration curve given in Figure 3.1 so that mass balance could be determined with a higher degree of confidence.*			

4.1 Mushroom Preparation and Pretreatment

The Mushroom Preparation and Pretreatment Procedure details the steps that were taken to clean, chop, dry, pretreat and document the mushrooms used in experimentation. After the mushrooms have been properly prepared and dried thoroughly, the mushrooms were pretreated with sodium hydroxide (NaOH), a step that was expected to increase the adsorption abilities of the mushrooms [5].

The initial pretreatment methodology was based on *Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: A comparative study* by R. Vimala and N. Das. In this study, the pretreatment process involves boiling the prepared mushrooms in 0.5 M NaOH solution for 15 minutes [5]. However, early trials of this method produced pretreated mushrooms that had the appearance of being burnt after drying and possibly behaved as activated carbon instead (see Figure 4.2). Due to this problem, the amount of time for boiling was reduced to 5 minutes yet the same issue persisted. Therefore, the method was changed such that the 0.5 M NaOH solution was brought to a temperature of 100℃ and a stir bar was introduced so that the mushrooms would not settle to the bottom of the beaker. After the 5-minute boiling period, the pretreated mushrooms where then filtered and rinsed two times using deionized water. As a result of the new pretreatment procedure the mushrooms retained their initial color and consistency that is likened to how the mushroom looked before pretreatment (see Figure 4.1). The full pretreatment procedure can be found in Appendix A-1.

Figure 4. 1: Modified Pretreatment Mushrooms

Figure 4. 2: Original Pretreatment Mushrooms

4.2 Experimental Matrix Development

The experimental matrix is shown in Table 4.2 below. Three replicates of ten fungi mass variations, along with three control samples, per experiment, were tested. The three controls are as follows: 1000 mg of pretreated mushroom biomass with 50 mL of distilled water, 50 mL of 1000 mg Pb stock solution with no biomass, and 1000 mg of pretreated mushroom biomass with no water or solution. After testing the mushroom biomass independently of the solution, it was determined that copper was an existing element in the mushrooms. This discovery led the team to remove copper from the experiment and replace it with additional testing of the lead stock solution. Because copper is present in the mushrooms, the copper's origin during the testing process cannot be determined and would result in additional error.

Table 4. 2: Pb Experimental Matrix

Type	Pretreated Mushroom Mass	Replicates	Initial Pb Concentration	Sample Volume
	(mg)	--	*ppm*	*mL*
Adsorption Samples	100	3	1000	50
	200	3	1000	50
	300	3	1000	50
	400	3	1000	50
	500	3	1000	50
	600	3	1000	50
	700	3	1000	50
	800	3	1000	50
	900	3	1000	50
	1000	3	1000	50
Control	0	1	1000	50
	1000	1	0	0
	1000	1	DI Water	50

4.3 Adsorption Using Lead 1000 ppm Solution with Original Pretreatment Method

The mushroom samples that were used in this first experiment underwent a pretreatment process that overcooked the mushrooms, referred to as the original pretreatment method. The results of this experiment are shown in Figure 4.3 and Table 4.3 below.
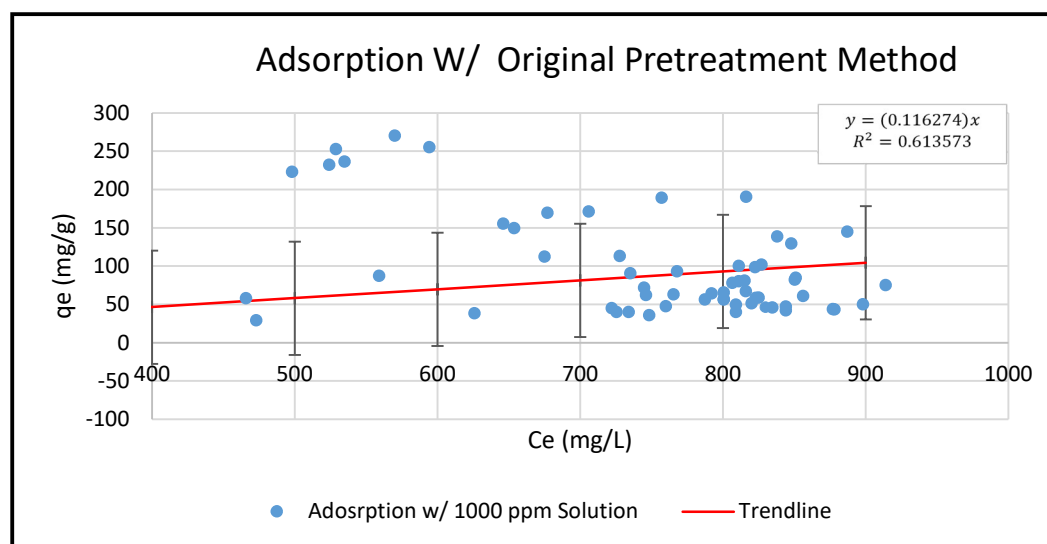
Figure 4. 3: Isotherm - -Original Pretreatment

Table 4. 3: Original Pretreatment % Removal

Procedure Type	Percent Removal (%)
Original Pretreatment	78.09

The original method used to pretreat the mushroom biomass showed a wider range of adsorption. Since the results in Figure 4.3 show a wider range of sorption across the range of equilibrium concentration values (C_e) it was deemed to behave more like activated carbon. This conclusion was made based on how the plot above shows a distinct linearity and less clustering around 800 ppm.

4.4 Adsorption Using Lead 1000 ppm Solution with No Pretreatment Method

To test the effect of pretreatment on the adsorption experiment, another trial was conducted on only dried mushroom biomass. This data can be seen in Figure 4.4 and Table 4.4 below. Full results of this experiment can be found in Appendix B-3.
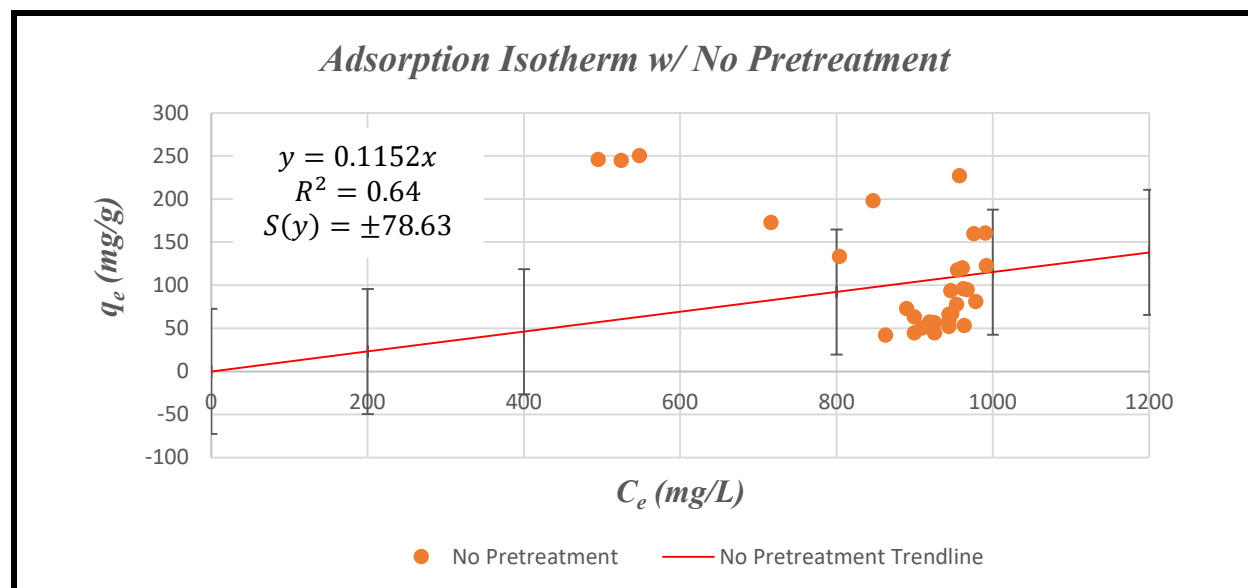
Figure 4. 4: Isotherm - No Pretreatment

Table 4. 4: No Pretreatment % Removal

Procedure Type	Percent Removal (%)
No Pretreatment	88.44
Partitioning Coefficient (K)	**Partitioning Coefficient Error**
$0.1152\dfrac{L}{g}$	$\pm\,0.0179\dfrac{L}{g}$

There is some uncertainty about how this experiment contradicts other studies that showed that pretreatment improves adsorption. However, this experiment appears to indicate the opposite.

4.5 Adsorption Using Lead 1000 ppm Solution with Modified Pretreatment Method
Once the pretreatment procedure was refined, an experiment was conducted with this modified procedure under the same conditions. The data is presented in Figure 4.5 and Table 4.5 below. Full results for this experiment can be found in Appendix B-2.
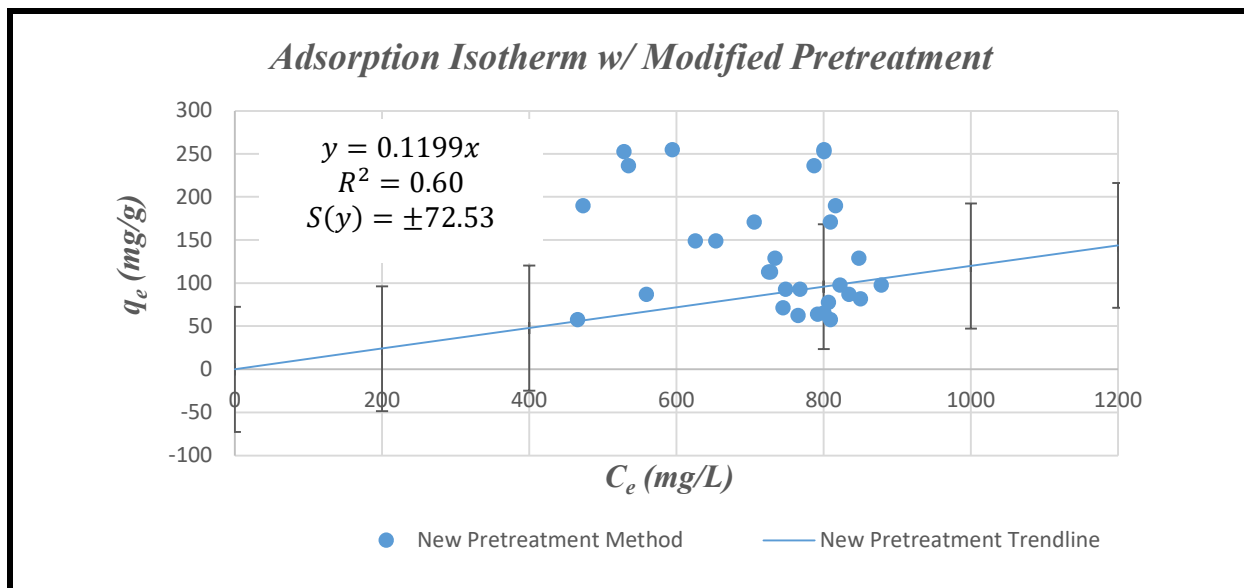
Figure 4. 5: Isotherm - Modified Pretreatment – 1000 ppm

Table 4. 5: Modified Pretreatment % Removal- 1000 ppm

Procedure Type	Percent Removal (%)
Modified Pretreatment	74.22
Partitioning Coefficient (K)	**Partitioning Coefficient Error**
$0.1199\dfrac{L}{g}$	$\pm\,0.0160\dfrac{L}{g}$

4.6 Final Results for Adsorption Experiment Using New Pretreatment Method
Data from both experiments using the new pretreatment procedure are given in Appendix B-2 and Appendix B-4 for Pb stock solutions of 1000 and 400 ppm, respectively. These results are shown in Figure 4.6. Figure 4.6 was used to estimate the partition coefficient via linear regression for the adsorption of Pb in mushrooms.
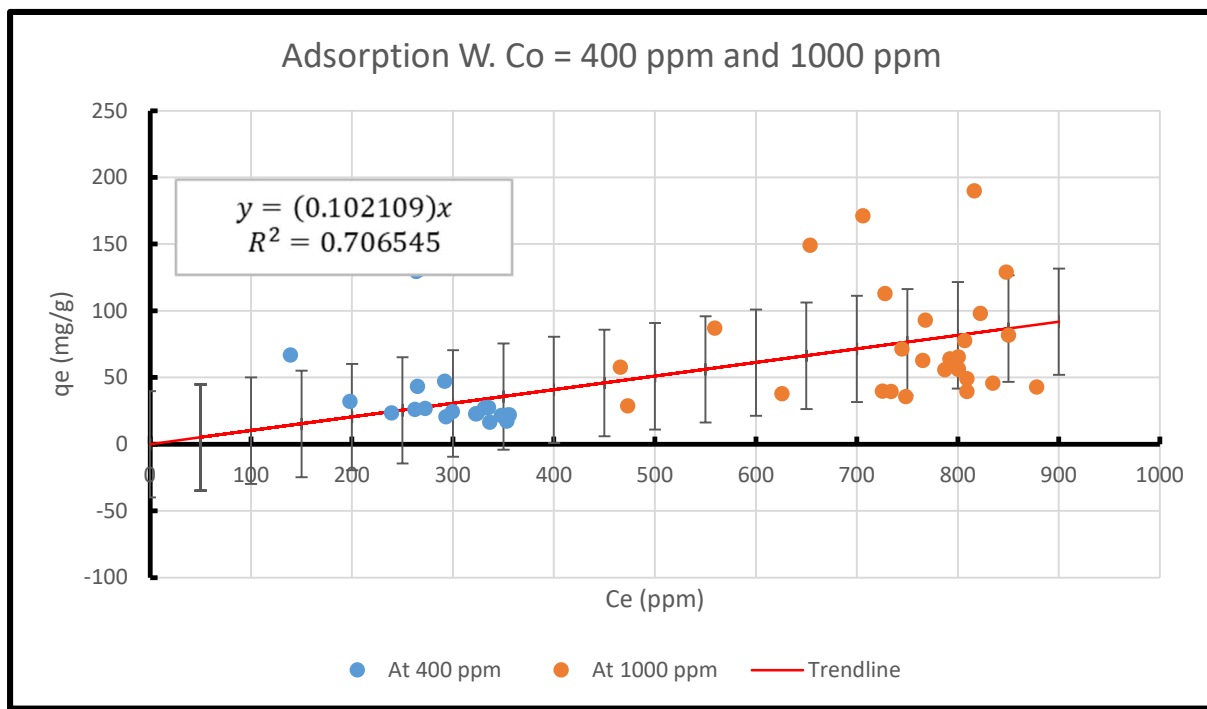
Figure 4. 6: Isotherm - Modified - 400 ppm and 1000 ppm

The data that was born out of this study indicates that a non-pretreated mushroom does have a higher percent removal, however, further study into this matter is recommended. Since there was uncertainty regarding the percent removal of lead, the system design aspect of the project incorporated the pre-treatment data because other research had indicated the validity of the use of a pretreatment.

The summary of adsorption partitioning coefficient and the percent removal is summarized in Table 4.6. Full data, regarding percent removal can be found in Appendix C.

Table 4. 6: Modified Pretreatment % Removal – 400 ppm and 1000 ppm

Procedure Type	Percent Removal (%)
Modified Pretreatment	74.50
Partitioning Coefficient (K)	**Partitioning Coefficient Error**
$0.1021\frac{L}{g}$	$\pm\,0.0096\frac{L}{g}$

5.0 Preliminary Design of a Treatment System

5.1 Evaluation of Alternative Systems

Research has been done to determine a potential and suitable large-scale treatment system. Multiple studies have demonstrated that the removal of copper (Cu) and lead (Pb) is possible using different species of fungi, and further studies have used a stirred tank reactor or a fixed bed adsorption column as a treatment system on a smaller scale.

For a scaled-up system, the following parameters are significant: initial concentration of the influent, pH, temperature, the amount of biomass per volume of liquid are important for removal efficiency and the rate of sorption. [5,6]. Since the experiments in this study have been carried out as a batch experiment, data obtained are not applicable to a column or fixed bed or CSTR and would better fit a batch reactor. Further experimentation is required using a fixed bed column at a laboratory scale in order to be able to design a fixed bed column treatment system for a commercial or industrial scale. Because this study did not perform any sorption rate experiments, an adsorption column could not be designed. Therefore, a simple single batch reactor was designed.

5.2 Design of System

The batch reactor has been designed based on the linear isotherm that was obtained from the experiment. A reactor capable of treating 1000 gallons (3785.4 L) of Pb-contaminated wastewater per batch will be designed. The maximum contamination limit (MCL) of lead from mine waste, represented by Ce, is set by the EPA at 0.6 mg/L [7].

5.2.1 Equations

The following equations were used in the scale up, from the experiment to the hypothetical treatment system design. Equation 5.1 below indicates the mass of solute adsorbed per mass of adsorbent. Equation 5.2 below is solved for the required mass rate of biomass (kg).

Equation 5. 1: Solute Adsorbed Per Mass of Adsorbent [8]

$$q_e = k_d C_e$$

q_e: Equilibrium Adsorption Value (mg Pb/g biomass)

C_e: Equilibrium Solution Concentration (mg Pb / L water)

k_d: Partitioning Coefficient (L water/g biomass)

Equation 5. 2: Required Mass Rate of Adsorbent [8]

$$m = \frac{V(C_0 - C_e)}{q_e}$$

q_e: Equilibrium Adsorption Value (mg Pb/g biomass)

C_e: Equilibrium Solution Concentration (mg Pb/L water)

C_0: Initial Solution Concentration (mg Pb/L water)

V: Volume (L water)

m: Mushroom biomass (g biomass)

5.2.2 Hypothetical Design
The hypothetical design of the batch reactor based on the obtained isotherm data is summarized below. Table 5.1 shows the partitioning coefficient (K), the maximum contamination limit (C_e), the mass of solute adsorbed per mass of adsorbent (q_e), the initial concentration of solute (C_0), the volumetric rate of solute (V), and the required mass rate of adsorbent (m).

The mass of solute adsorbed per mass of adsorbent (qe) was calculated using Equation 1, and the required mass rate of adsorbent (m) was calculated using Equation 2. The required volume rate of NaOH and rinsing deionized water has been determined by multiplying the required biomass by the rate of 50 mL of NaOH or rinsing water per 14 grams of mushroom.

Table 5. 1: Daily Fungi Mass to Adsorb Lead for from 1000 gpd

K	C_e	q_e	C_0	V	m
(mg Pb/L Water)	(mg/L)	(mg/g)	(mg/L)	(L)	(kg)
0.1152	0.6	0.06912	60	3785.4	3253.07
0. 1152	0.6	0. 06912	50	3785.4	2705.42
0. 1152	0.6	0. 06912	40	3785.4	2157.77
0. 1152	0.6	0. 06912	30	3785.4	1610.11
0. 1152	0.6	0. 06912	20	3785.4	1062.45
0. 1152	0.6	0. 06912	10	3785.4	514.80

Based upon these results, the design will be created for an input wastewater contaminant concentration of 60 mg/L.

Figure 5.1 below shows how the sorption process is carried out in one batch reactor with an axial hydraulic impeller. The first step is the filling step, where the batch reactor is filled with a lead contaminated solution, and usually takes up about 25% of the treatment cycle duration. The next step is the reacting step, where the contact between the mushroom and the contaminated solution happens and usually takes up about 35% of the treatment cycle duration, depending on a set residence time. The hydraulic impeller is a flexible impeller, and it can be moved up and down in order to allow the hydraulic filter to enter the treatment system. The settling and decanting process usually takes up about 40% of the treatment cycle duration [9]. A complete procedure for the sorption experimental system is provided in Appendix A-4.
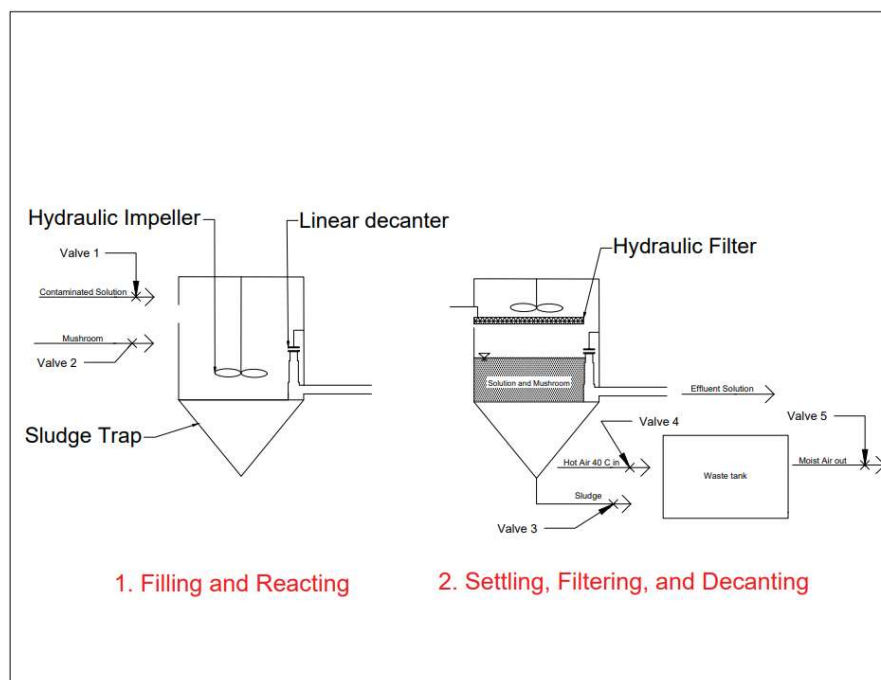
Figure 5. 1: Batch Reactor Treatment System

The batch reactor shall be constructed of plastic. Plastic does not erode when it is exposed to corrosive solutions such as acids and bases.

6.0 Impact Analysis

Mine waste can be extremely toxic, and in the case of accidental mine spills, the cost of remediation can be very high when using traditional treatment methods. Because of this, there is an emphasis on research that may lead to legitimate alternative treatment systems, such as the use of fungi to absorb metal pollutants.

Environmental impacts would present in the way that mine wastewater is treated, how mine spills are remediated, or in several other ways (i.e. inspiring similar research, on site mine water management, etc.). Mine waste is very harmful to the environment, particularly when major waterways are contaminated, as the pollutants are quickly transported across large distances and can accumulate up the biological food chain. The use of fungi as a biosorbent may provide an effective treatment method to reduce contaminants in the environment, while also reducing the need for chemical treatment options.

Public impacts would present in the safety of the land around them. For communities near old or current mining site, a biotechnology such as mushrooms used as a biosorbent is both a sustainable and environmentally healthier alternative to traditional remediation methods. These types of treatment methods may pose less risk to the families who live nearby.

Economic impacts would present as possible lower cost to clean-ups or treatment of wastewater because a common mushroom, such as *Agaricus bisporus*, is readily available. Because water is used in such high demand in developed cities, the cost of water treatment can be extremely high.

Therefore, there is interest in finding alternative treatment options for water treatment. Fungi can be grown quickly, in large quantities, and may be a cost-effective alternative treatment method for some remediation efforts.

7.0 Summary of Engineering Work

The final project had many changes from what was originally proposed. During the proposal stage of this project, two different teams were studying fungi and were combined into one for the design phase. Additionally, accessibility and timing problems resulted in major changes from the original scope and plan. These changes are discussed in the following sections.

7.1 Changes in Scope

Several major changes have been made to the scope of the project. The team had difficulty getting access to the NAU Biology's Mycology laboratory to grow fungi. After suffering from many time delays, the team made the decision to use common mushroom species that could be easily purchased from a local grocery store. This changed the entirety of the second task from Task 2: Cultivate Fungi to the new task, Task 2: Obtain Mushroom. Adjustments were made to the third task such as adding experimental procedures and methodologies, preparing metal solution samples, and creating data sheets, all of which were designed to save time once the team had access to the Environmental Engineering Lab.

The next major change occurred during the initial phase of Task 4: Experimentation. Previously, the team had chosen to use copper for experimentation, yet upon preliminary testing, it was discovered that the mushrooms that were chosen for use had large amounts of copper present in the biomass prior to testing. This led the team to decide to only move forward with the lead experiments and not pursue the copper experiments. This decision, as well as other knowledge the team recently learned about mushroom pretreatment and how to operate the XRF, led to the expansion of Task 4. The task was renamed to Task 4: Perform Experiments.

The following task, Task 5: Data Analysis was adjusted as well. The subtasks initially included testing the water solutions, testing the mushrooms, and creating adsorption isotherms. They were changed to just two subtasks, Task 5.1: Isotherm Plot Creation and Task 5.2: Adsorption Coefficient. Mushroom testing was omitted after the first experiment, as it was found that the XRF had difficulty testing the mushroom sample due to the very small masses of mushroom that were used. The tasks were adjusted and renamed to better portray the reality of the first experiment that had been performed.

An additional task was added as Task 6: Preliminary Design of a Treatment System. This task involves the design of a simple treatment system based on data obtained from the results of the adsorption isotherm experiments. This task was added as it was specifically requested by the client to further the implementation of the research.

The proposed Gantt chart and actual Gantt chart for the project is shown in Appendix D. These show the scope of the project as well as the schedule. The major changes discussed above are evident, yet all milestones were met on generally the same timeline.

7.2 Changes in Schedule

As discussed above, the first major problem was the delay in accessing the laboratories. The biology lab could only be accessed once the team had training and been granted access; however, communication was very slow with the mycologist. This eventually pushed the schedule back so much that the team chose to change the scope of the project, as mentioned previously, and avoid growing fungi in general.

A lab binder was then required by the Environmental Engineering Laboratory to be compiled by the team before entering the lab space. This task lasted longer than expected as well due to slow communication and similar issues as the biology lab. The lab binder had become so significant that the team adjusted the 30% milestone to be the completion of the lab binder instead of fungi growth, given that task had been removed. No further other issues significantly affected the schedule of the project.

Failing to access the biology lab changed major sections of the project, specifically switching to store-bought mushrooms from grown fungi. The delay in entering the Environmental Engineering lab did not have major impacts on the overall project, as the team was able to make up the lost time relatively quickly, once lab access had been granted. The team worked efficiently together to complete tasks more quickly than originally anticipated.

8.0 Summary of Engineering Costs

8.1 Changes in Staffing

The team underwent major adjustments during the project. Initially, the project had only three team members. This was adjusted to five team members at the beginning of the experimental phase. The original hours of the three staff members (Engineer, Senior Engineer, and Lab Technician) had to be increased to account for the expected 150 hours per team member. The proposed hours distribution between the staff is shown in Table 8.1 below. This addition of two team members helped lighten the workload for each team member, allowing quality of work to remain a top priority. Each team member put in significant effort, helping the project get back on track after the many adjustments and delays that took place. The proposed hours for the project and the actual hours log are shown below.

Table 8. 1: Proposed Hours

Summary of Proposed Hours									
Team Member	Nolan			Masad			Yue		
Staff	Lab Tech	ENG 1	SENG	Lab Tech	ENG 1	SENG	Lab Tech	ENG 1	SENG
Total Hours Per Position	25	75	50	25	75	50	25	75	50
Total Hours Per Person	150			150			150		

Table 8. 2: Actual Hours Log

Summary of Hours Log															
Team Member	William			Nolan			Masad			Danielle			Yue		
Staff	Lab Tech	ENG 1	SENG	Lab Tech	ENG 1	SENG	Lab Tech	ENG 1	SENG	Lab Tech	ENG 1	SENG	Lab Tech	ENG 1	SENG
Total hours per Position	38.5	74.5	35.5	44	71.75	33.5	44	57.5	43.5	43	68.75	32.5	40.5	68.5	33.5
Total Hours per Person	148.5			149.25			145			144.25			142.5		

8.2 Final Costs

The proposed and final costs are shown in the following tables and include both theoretical and actual dollar amounts. These costs include the cost of personnel and supplies. Most of the costs come from the personnel, as very few supplies were needed for the project. The costs were also based on actual hours of work by the team members, which is shown in the hours log in the table above. There are three classifications being filled by five team members who are applying themselves in different aspects of the project. In actuality, the PPE costs were zero as the PPE was included in the lab use fee. The team was also able to use the XRF to test liquid solutions and therefore did not outsource samples to other labs for analysis.

Table 8. 3: Proposed Costs

Proposed Cost of Engineering Services				
1.0 Personnel	Classification	Hours	Rate, $/hr	Cost ($)
	SENG	150	125.58	18837
	ENG I	150	77.28	11592
	LAB	150	32.92	4938
	Total personnel cost			35367
2.0 Supplies	2.1 NAU lab time	15 days	$100/day	1500
	2.3 PPE	5	$20/Item	100
3.0 Subcontract				$150
4.0 Total				37117

Table 8. 4: Actual Costs

Cost of Engineering Services				
1.0 Personnel	Classification	Hours	Rate, $/hr	Cost ($)
	SENG	178.5	125.58	22416
	ENG I	341	77.28	26352
	LAB	210	32.92	6913
	Total personnel cost			55681
2.0 Supplies	2.1 NAU lab time	15 days	$100/day	1500
	2.2 Mushroom	15 boxes	$4/box	60
	2.3 PPE	0	$20/Item	0
3.0 Total				57241

The final cost of the project is significantly over budget compared to the original projected cost. The proposed cost was $37,117. The actual final cost of the project is $57,241 as shown in Table 8.4 above. This change is a result of several adjustments made to the project that limited outside resources in favor of what was available through the Engineering department. The difference in hours worked is the major difference between the projected and final cost, which resulted from the addition of two team members.

9.0 Conclusion

The objective of this project was to analyze the feasibility of fungi to remove heavy metals from mine wastewater. It was shown that *Agaricus bisporus*, better known as the common white button mushroom, could absorb a significant amount of lead from 1000 ppm and 400 ppm lead stock solutions with percent removals ranging from 74% to 88%. The lead concentrations were read with an XRF with reasonable accuracy after creating a calibration curve from known concentration points. With the data, an adsorption isotherm was made, a graphical interpretation of the ability of the *Agaricus bisporus* to absorb lead from synthetic wastewater. A treatment system was designed to show the applicability of this experiment to a real-world scenario, specifically the possibility of using *Agaricus bisporus* for mine wastewater treatment, mine accident remediation, or similar scenarios. Because lead is a very toxic substance, we believe this study may be particularly important to providing insight into alternative methods of metal pollution removal from water sources.

10.0 References

[1] United States Environmental Protection Agency, "Costs of Remediation at Mine Sites," Office of Solid Waste, Washington, DC, 1997.

[2] N. P. Lotliker, S. R. Damare, R. M. Meena, P. Linsy and B. Mascarenhas, "Potential of Marine-Derived Fungi to Remove Hexavalent Chromium Pollutant from Culture Broth," Indian Journal of Microbiology, vol. 58, no. 2, pp. 182-192, 2018.

[3] C. McLeod, D. Hammond and M. Umlor, "Remediation of E. Coli Contaminated Surface Water in," Flagstaff, 2020.

[4] M. E. Mahmound, A. A. Yakout, H. Abdel-Aal and M. M. Osman, "Immobilization of Fusarium verticillioides Fungus on Nano-Silica (NSi-Fus):A novel and Efficient Biosorbent for Water Treatment and Solid Phase Extraction of Mg(II) and Ca(II)," Bioresource Technology, vol. 134, no. 1, pp. 324-330, 2013.

[5] R. Vimala and N. Das, "Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: A comparative study," Journal of Hazardous Materials, pp. 376-382, 2009.

[6] H. Hindarso, S. Ismadji, F. Wicaksana, M. and N. Indraswati, "Adsorption of Benzene and Toluene from Aqueous Solution onto," Journal of Chemical and Engineering Data, vol. 46, no. 4, 2001.

[7] Prem Kumar Gautam, Deveshwari Singh, and Seema Bhadauria, "Biosorption of Lead (II) from Aqueous Solution by Agaricus bisporus," Nature Environment and Pollution Technology, vol. 11, no. 3, pp. 493-496, 2012.

[8] M. L. Davis, Water and Wastewater Engineering: Design Principles and Practice.

[9] Mining Industry Profile: Copper | US EPA ARCHIVE DOCUMENT, Environmental Protection Agency (EPA).

Appendix A: Procedures

Appendix A-1: XRF Detection Limit Procedure
CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Wastewater Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Created by: William Bain and Nolan Maxwell

Procedure: XRF Detection Limit

Application: Determine Copper and Lead Detection Limit for Wastewater Sample Solution

Summary: The purpose of this procedure is to identify the XRF detection limits for lead and copper in liquid solutions. The copper chloride is a powder, while the lead acetate is a stock solution. Dilute solutions will be created for the copper chloride and lead acetate with a different series of steps.

1.0   Equipment

   (a)   200 mL Beaker
   (b)   1000 mL Beaker
   (c)   Analytical Scale
   (d)   Fume Hood
   (e)   Stir Rod
   (f)   XRF

2.0   Reagents and Materials

   (a) Copper Chloride

   (c) Lead Acetate Solution at 386 mg/mL

3.0   XRF Detection Limit for Copper Procedure
1.   Add 100 mL of distilled water to a 200 mL beaker.
2.   Measure out 0.21 g of copper chloride on a scale.
3.   Add the copper chloride to the beaker under the fume hood and stir with a stir rod until the powder is fully mixed into the water.
4.   Test the copper chloride solution with the XRF device.
   a.   Add a small amount of the solution to the sample container
   b.   Cover the sample with a specialized film and screw on the lid
   c.   Place filled and sealed sample container in lead plated box to be measured by the XRF device

5.   Test the copper chloride solution with the XRF device.
   a.   Add a small amount of the solution to the sample container

       b. Cover the sample with a specialized film and screw on the lid
       c. Place filled and sealed sample container in lead plated box to be measured by the XRF device

6. If the concentration of the solution is within range for the XRF device
   a. If the solution is not within range because the concentration is too low, repeat Steps 2 through 5, adding 0.21 g of copper chloride to the solution repeatedly until the XRF device produces a reading.
   b. If the solution is within range, dilute the solution in half and retest until a non-detect is generated

7. Once a reading has been generated by the device, retest the same solution multiple times to check for consistency in the XRF device's ability to read the copper chloride in à liquid sample.

8. The lowest concentration of copper chloride that the XRF is capable of reading is the XRF detection limit for copper chloride.

    4.0   XRF Detection Limit for Lead Procedure

1. 4.36 mL of the 360 g/L lead acetate solution will be added to a 1000 mL beaker where the rest of the container should be filled with DI water.
   a. This is stock solution is highly concentrated, therefore small amounts are needed to reach desired concentrations
   b. This amount will be used for dilutions if necessary

2. Test the lead acetate solution with the XRF
   a. Add a small amount of the solution to the sample container
   b. Cover the sample with a specialized film and screw on the lid
   c. Place filled and sealed sample container in lead plated box to be measured by the XRF device

3. If the concentration of the solution is within range for the XRF device and produces a reading move on to Step 4 to dilute the solution. The solution should be diluted until the XRF device can no longer produce a reading, at this point the detection limit could be approximately the last viable reading.

4. Add 50 mL of distilled water to the beaker, stir the solution to mix, and repeat Step 3.

Appendix A-2: Mushroom Preparation and Pretreatment Procedure

CENE 486 Capstone Project—Adsorption of Copper and Lead
from Mine Wastewater Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Created by: Sara Danielle Gallaher

Procedure: Mushroom Procedure and Pre-Treatment

Application: Prepare a mushroom biomass to be for adsorption experiments

Summary: Before the mushrooms can be used in the experiment, they must be properly
             prepared and pretreated. Mushrooms should be cut into even pieces and dried.
             Additionally, to break down cell walls in the mushroom, it is pretreated with
             NaOH to increase sorption and removal efficiency.

1.0   Equipment

      (a)   10 Evaporating Dishes
      (b)   1 Knife to chop up mushrooms
      (c)   Drying Oven
      (d)   Hot Plate
      (e)   Scales
      (f)   1 Pot to be used with the hot plate
      (g)   Filter Paper - #691
      (h)   1 1000 mL Erlenmeyer Flask
      (i)   Fume Hood
      (j)   DI Squirt Bottle

2.0   Reagents and Materials

      (a)   50 g of Agaricus Bisporus (White Mushroom)
      (b)   500 mL of 0.5 M NaOH

3.0   Mushroom Preparation Procedure

1. Once mushrooms have been purchased, they must first be washed and scrubbed of any dirt
    and/or other contaminants that may be present on the mushrooms.
2. Whole mushrooms should be chopped into small pieces using a knife. Pieces should be
    approximately 1 cm across so that once they are dried, they are not too small to impede
    filtering in later steps.

3. Prior to drying, weigh the raw chopped mushroom and record data in the Mushroom Preparation and Pretreatment Data Sheet
4. Approximately 50 grams of wet mushroom biomass should be placed in each evaporating dish to be placed in the drying oven in batches
5. Set the oven temperature to 40°C for 24 hours to allow all moisture to evaporate from the biomass
6. Once dried, mushrooms should be weighed again and recorded in the Mushroom Preparation and Pretreatment Data Sheet to account for water loss and prepare for further testing and predicting the amount of raw mushroom biomass needed for all samples.

   4.0   Mushroom Pre-Treatment Procedure

   Note: The prepared biomass from above should be pretreated with sodium hydroxide (NaOH) to increase the sorption rate of the mushroom biomass.

1. Approximately 500 mL of 0.5 M NaOH solution should be added to 20 grams of dried biomass
2. This combination will be boiled on a hotplate under the fume hood at 100°C for 5 minutes and then left to cool for 10 minutes.
3. Once the biomass and NaOH have cooled, it is to be filtered to separate the biomass from the NaOH over a large beaker.
4. While the biomass is still on the filter, it should be rinsed briefly with deionized water from a squirt bottle to remove any remaining basic solution. Additionally, the biomass should be soaked in 500 mL of DI water, separated, then soaked again. This process should be conducted at least two times.
5. The biomass should be dried over 24 hours, then it will be ready for use in sample vials

Appendix A-3: Adsorption Experiment Procedure

CENE 486 Capstone Project—Adsorption of Copper and Lead
from Mine Wastewater Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Procedure: Mushroom Adsorption Setup
Application: Generate Adsorption Isotherm Data

Summary: The aim of this experiment is to quantitatively show the adsorption capabilities of mushrooms when used as a biosorbent. This is accomplished by creating a mine waste water sample containing copper and lead and allowing the solution to adsorb to coarsely processed mushroom that was pretreated with sodium hydroxide. The adsorption of copper and lead is measured using an XRF by analyzing the change in metal concentration in both the retained liquid and mushroom biomass. An adsorption isotherm will be constructed from the collected data allowing for further insight into the adsorption qualities of white button mushrooms.

1.0   Equipment
- (a) 36 Glass Reaction Vials (60 mL)
- (b) 50 mL Volumetric Flask w/ Filter Funnel
- (c) Filter Paper
- (d) Rubber Vacuum Hose
- (e) Analytical Balance
- (f) Rotary Shaker Table
- (g) Weight Paper
- (h) PPE (gloves, coat, goggles, etc.)
- (i) Fume Hood
- (j) 36 XRF Sample Cups

2.0   Reagents and Materials
- (a) 15 g of Pre-Treated Agaricus Bosporus (White Button Mushroom)
- (b) 1000 ppm Copper Mine Waste Solution (2L)
- (c) 1000 ppm Lead Mine Waste Solution (2L)

3.0   Procedure
3.1   Mushroom Adsorption of Copper

1. In order to create the samples for the Copper adsorption experiment weigh out ten triplicate masses of mushrooms at the following weights from the 15 g of dried pretreated mushrooms (prepared using the Mushroom Preparation and Pretreatment Procedure): 100 mg, 200 mg,

300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, and 100 mg. There should be 30 mushroom samples in total. In addition to the 30 samples, weight out three 1000 mg mushroom samples for the purpose of creating three control samples.

2. Obtain 33 glass reaction vials, label each vial by sample number, and pour each mushroom sample into each corresponding vial. The sample numbers will be labeled by metal content, mushroom weight, and replicate number (i.e. Cu-XXX-01, CuXXX-02, etc.)

3. While in the fume hood pour 50 mL of the 1000 ppm Copper Mine Waste Solution into each vial using a funnel and a volumetric flask. Cap each vial.

4. Place the three 10 mg samples into three glass reaction vials and fill each vial with 50 mL of distilled water. Cap each vial. These three samples will serve as the control for this experiment.

5. Place all vials on a Rotary Shaker Table for 24 hours.

6. Pipette approximately 4 mL of liquid from the top of each vial and put into a labeled XRF sample cup that is labeled the same way as the samples in each vial.

7. Filter each mushroom sample separately by using a suction flask and filter funnel with filter paper. DO NOT COMBINE ALL FILTERED LIQUIDS. The remaining filtered liquid will be discarded into the lead/copper waste bucket.

8. Place each mushroom sample in an evaporating dish and place in drying furnace at 40°C for 24 hours. Each sample dish will be labeled with the corresponding label in which the sample came from.

9. Prepare the XRF sample cups by first capping one side of the cup with a plastic liner, then fill the sample cup with the liquid sample retained in the sample flask. Cap the XRF sample cup using another plastic liner. Repeat for each filtered mushroom sample. Store remaining liquid samples in separate containers for potential repeated analysis.

10. After 24 hours remove mushrooms from the drying furnace, allow to cool for 30 minutes. Place each sample into a XRF sample cup and then cap each sample. At this point there should be a total of 33 mushroom samples including the three control samples.

3.2 Mushroom Adsorption of Lead

1. In order to create the samples for the lead adsorption experiment weigh out ten triplicate masses of mushrooms at the following weights from the 15 g of dried pretreated mushrooms (prepared using the Mushroom Preparation and Pretreatment Procedure): 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, and 100 mg. There should be 30 mushroom samples in total. In addition to the 30 samples, weight out three 1000 mg mushroom samples for the purpose of creating three control samples.

2. Obtain 33 glass reaction vials, label each vial by sample number, and pour each mushroom sample into each corresponding vial.

3. While in the fume hood pour 50 mL of the 1000 ppm Lead Mine Waste Solution into each vial using a funnel and a volumetric flask. Cap each vial.

4. Place the three 10 mg samples into three glass reaction vials and fill each vial with 50 mL of distilled water. Cap each vial. These three samples will serve as the control for this experiment

5. Place all vials on a Rotary Shaker Table for 24 hours.

6. Pipette approximately 4 mL of liquid from the top of each vial and put into a labeled XRF sample cup that is label the same way as the samples in each vial.

7. Filter each mushroom sample separately by using a suction flask and filter funnel with filter paper. DO NOT COMBINE ALL FILTERED LIQUIDS. The remaining filtered liquid will be discarded into the lead/copper waste bucket.

8. Place each mushroom sample in an evaporating dish and place in drying furnace at 40°C for 24 hours. Each sample dish will be labeled with the corresponding label in which the sample came from.

9. Prepare the XRF sample cups by first capping one side of the cup with a plastic liner, then fill the sample cup with the liquid sample retained in the sample flask. Cap the XRF sample cup using another plastic liner. Repeat for each filtered mushroom sample. Store remaining liquid samples in separate containers for potential repeated analysis.

10. After 24 hours remove mushrooms from the drying furnace, allow to cool for 30 minutes. Place each sample into a XRF sample cup and then cap each sample. At this point there should be a total of 33 mushroom samples including the three control samples.

Appendix A-4: Operation of Batch Reactor Procedure

Environmental W.W. Engineering

Created by: Masad Alyahya

Procedure: Operating a Sequencing Batch Reactor Treatment System

Application: Operate a sequencing batch reactor treatment system to remediate contaminated Lead (Pb) solution using a mushroom biomass.

Summary: The treatment cycle duration of an industrial waste is usually between 4.0 – 24 hours. The filling process usually represents the 25% treatment cycle duration. The reacting process represents 35%; meanwhile, settling and decanting is 35%, and idling is 5% of the treatment cycle duration [9]. The idling step is only needed when more retention time is needed. The maximum contaminated limit of Lead (Pb) is 0.6 mg/L according the Environmental protection Agency (EPA) [7].

1.0 **Equipment**

(a) Pretreated Mushroom

(b) Batch Reactor

(c) Linear Decanter

(d) Filter

(e) Waste Tank

2.0 **Reagents and Materials**

(a) Mass of Agaricus Bisporus (White Mushroom) (g)

3.0 ***Contained Lead (Pb) Sorption Procedure***

Note: All the parameters mentioned in the procedure below is based on a demand of 1000 gpm, and an initial concentration of 60 mg/L.

1. 1000 gpm of contaminated solutions shall be added to the batch rector (filling).

2. After filling the batch reactor with the contaminated solution, 3674.603 kg/d of the pretreated mushroom shall be added to the treatment to adsorb the Lead (Pb) contamination for about 8.5 hours by opening Valve 5 (Reacting).

3. Since the biomass does not settle out, a filter shall be used to settle out the biomass. While the biomass is being settled and filtered, the contaminated solution shall be decanted by the linear decanter as a treated solution. This process takes about 8.5 hours.

4. The sludge shall be moved to the waste tank by opening Valve 6.

5. The sludge shall be exposed in the waste tank to a hot air at 40 ℃ for 24 hours to dry

Appendix B: Data Sheets

Appendix B-1: Original Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Names: William, Danielle, Yue, and Nolan
Location: NAU EGR 245

Mushroom Sorption Data Sheet
--Lead (Liquid)--

Sample #	Mushroom Biomass (mg)	$[C]_o$ (ppm)	$[C]_f$ (ppm)	C_e (ppm)	q_e (mg/g)
PB100-1	111.7	1000	502	498	222.92
PB100-2	112.8	1000	476	524	232.27
PB100-3	105.4	1000	430	570	270.40
PB200-1	200	1000	243	757	189.25
PB200-2	207.9	1000	354	646	155.36
PB200-3	199.8	1000	323	677	169.42
PB300-1	300.9	1000	325	675	112.16
PB300-2	306.4	1000	113	887	144.75
PB300-3	302.2	1000	162	838	138.65
PB400-1	406.4	1000	265	735	90.43
PB400-2	406.2	1000	173	827	101.80
PB400-3	405.8	1000	189	811	99.93
PB500-1	504.7	1000	185	815	80.74
PB500-2	505.8	1000	189	811	80.17
PB500-3	502.4	1000	149	851	84.69
PB600-1	609.9	1000	86	914	74.93
PB600-2	608.8	1000	184	816	67.02
PB600-3	602.3	1000	254	746	61.93

Figure B. 1: Liquid Sample Data Sheet for Original Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Mushroom Sorption Data Sheet Continued
--Lead (Liquid)--

Sample #	Mushroom Biomass (mg)	$[C]_o$ (ppm)	$[C]_f$ (ppm)	C_e (ppm)	q_e (mg/g)
PB700-1	700.9	1000	175	825	58.85
PB700-2	703.8	1000	144	856	60.81
PB700-3	705.2	1000	177	823	58.35
PB800-1	802.1	1000	180	820	51.12
PB800-2	804.3	1000	240	760	47.25
PB800-3	801.4	1000	278	722	45.05
PB900-1	904.3	1000	170	830	45.89
PB900-2	902.1	1000	156	844	46.78
PB900-3	901.5	1000	102	898	49.81
PB1000-1	1000.6	1000	156	844	42.17
PB1000-2	1005.5	1000	123	877	43.61
PB1000-3	1000.3	1000	69	931	46.54
C1-H2O+M1000	1007.9	NA	ND		
C2-PB	--	1000	943.023		
C3-M1000	1003.6	NA	ND		

Figure B. 2: Liquid Sample Data Sheet for Original Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Mushroom Sorption Data Sheet Continued
--Lead (Mushroom)--

Sample #	ushroom Biomass (m	C_e (ppm)	q_e (mg/g)
PB700-1	700.9	3.63	0.26
PB700-2	703.8	3.28	0.23
PB700-3	705.2	3.42	0.24
PB800-1	802.1	3.2	0.20
PB800-2	804.3	2.09	0.13
PB800-3	801.4	1.84	0.11
PB900-1	904.3	2.56	0.14
PB900-2	902.1	2.98	0.17
PB900-3	901.5	2.52	0.14
PB1000-1	1000.6	3.18	0.16
PB1000-2	1005.5	2.77	0.14
PB1000-3	1000.3	2.54	0.13
C1-H2O+M1000	1007.9	NA	
C2-PB	--	1000	
C3-M1000	1003.6	NA	

$$q_e = \frac{C_e V}{m}$$

C_e: Equilibrium Concentration $(\frac{mg\ Pb}{L})$
q_e: equilibrium adsorption loading for mass of material adsorbed per mass of adsorbent having units of mg/g
V: Sample Volume (0.05 L)
m: Mass of Mushroom (g)

Figure B. 3: Biomass Sample Data Sheet for Original Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Names: William, Danielle, Yue, and Nolan
Location: NAU EGR 245

Mushroom Sorption Data Sheet
--Lead (Mushroom)--

Sample #	Mushroom Biomass (mg	C_e (ppm)	q_e (mg/g)
PB100-1	111.7	4.27	1.91
PB100-2	112.8	2.98	1.32
PB100-3	105.4	6.18	2.93
PB200-1	200	6.64	1.66
PB200-2	207.9	1.84	0.44
PB200-3	199.8	2.7	0.68
PB300-1	300.9	2.41	0.40
PB300-2	306.4	5.33	0.87
PB300-3	302.2	4.29	0.71
PB400-1	406.4	3.66	0.45
PB400-2	406.2	5.31	0.65
PB400-3	405.8	4.11	0.51
PB500-1	504.7	4.11	0.41
PB500-2	505.8	2.88	0.28
PB500-3	502.4	3.49	0.35
PB600-1	609.9	3.6	0.30
PB600-2	608.8	2.78	0.23
PB600-3	602.3	3.08	0.26

$$q_e = \frac{C_e V}{m}$$

C_e: Equilibrium Concentration $(\frac{mg\ Pb}{L})$
q_e: equilibrium adsorption loading for mass of material adsorbed per mass of adsorbent having units of mg/g
V: Sample Volume (0.05 L)

Figure B. 4: Biomass Sample Data Sheet for Original Pretreatment Method Experiment

Appendix B-2: Updated Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent				Environmental W.W. Engineering	
Names: William, Danielle, Yue, and Nolan Location: NAU EGR 245	*Mushroom Sorption Data Sheet* *--Lead (New Pre-Liquid)--*				

Sample #	Mushroom Biomass (mg)	$[C]_o$ (ppm)	$[C]_f$ (ppm)	C_e (ppm)	q_e (mg/g)
PB100-1	104.6	1000	471.22	528.78	252.76
PB100-2	116.4	1000	405.71	594.29	255.28
PB100-3	113.1	1000	465.16	534.84	236.45
PB200-1	206	1000	294.10	705.90	171.34
PB200-2	218.9	1000	346.26	653.74	149.32
PB200-3	214.6	1000	183.70	816.30	190.19
PB300-1	327.9	1000	152.15	847.85	129.28
PB300-2	320.3	1000	440.89	559.11	87.28
PB300-3	321.5	1000	272.26	727.74	113.18
PB400-1	412.1	1000	232.22	767.78	93.15
PB400-2	402.1	1000	534.31	465.69	57.91
PB400-3	418.7	1000	177.63	822.37	98.21
PB500-1	519	1000	149.73	850.27	81.91
PB500-2	518.1	1000	193.40	806.60	77.84
PB500-3	518.9	1000	255.27	744.73	71.76
PB600-1	617.5	1000	207.96	792.04	64.13
PB600-2	610.7	1000	199.47	800.53	65.54
PB600-3	608.3	1000	234.65	765.35	62.91

Figure B. 5: Liquid Sample Data Sheet for Updated Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent				Environmental W.W. Engineering	
Mushroom Sorption Data Sheet Continued *--Lead (New Pre-Liquid)--*					

Sample #	Mushroom Biomass (mg)	$[C]_o$ (ppm)	$[C]_f$ (ppm)	C_e (ppm)	q_e (mg/g)
PB700-1	705.3	1000	199.47	800.53	56.75
PB700-2	712.3	1000	199.47	800.53	56.19
PB700-3	700.7	1000	212.81	787.19	56.17
PB800-1	817.9	1000	190.97	809.03	49.46
PB800-2	823.4	1000	374.17	625.83	38.00
PB800-3	813.5	1000	527.03	472.97	29.07
PB900-1	920.8	1000	266.19	733.81	39.85
PB900-2	908.5	1000	165.50	834.50	45.93
PB900-3	906.3	1000	274.68	725.32	40.02
PB1000-1	1044.8	1000	251.63	748.37	35.81
PB1000-2	1016	1000	190.97	809.03	39.81
PB1000-3	1016.5	1000	121.82	878.18	43.20
C1-H20+M1000	1026.4	NA	ND		
C2-PB	--	1000	943.023		
C3-M1000	1032.3	NA	ND		

Figure B. 6: Liquid Sample Data Sheet for Updated Pretreatment Method Experiment

Appendix B-3: No Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Names: William, Danielle, Yue, and Nolan
Location: NAU EGR 245

Mushroom Sorption Data Sheet
--Lead (No Pretreatment)--

Sample #	Mushroom Biomass (mg)	[C]o (ppm)	[C]f (ppm)	Ce (ppm)	qe (mg/g)
PB100-1-u	109.3	1000	452.625	547.375	250.40	0.45262
PB100-2-u	107	1000	475.991	524.009	244.86	0.47599
PB100-3-u	100.6	1000	505.505	494.495	245.77	0.50551
PB200-1-u	207.2	1000	284.145	715.855	172.74	0.28414
PB200-2-u	213.8	1000	153.788	846.212	197.90	0.15379
PB200-3-u	210.6	1000	43.1079	956.892	227.18	0.04311
PB300-1-u	304.9	1000	24.6612	975.339	159.94	0.02466
PB300-2-u	308.7	1000	9.90387	990.096	160.37	0.0099
PB300-3-u	301.1	1000	196.831	803.169	133.37	0.19683
PB400-1-u	405.1	1000	45.5675	954.432	117.80	0.04557
PB400-2-u	403.6	1000	8.67409	991.326	122.81	0.00867
PB400-3-u	400.1	1000	39.4186	960.581	120.04	0.03942
PB500-1-u	510.5	1000	33.2697	966.73	94.68	0.03327
PB500-2-u	501.8	1000	38.1888	961.811	95.84	0.03819
PB500-3-u	505.4	1000	54.176	945.824	93.57	0.05418
PB600-1-u	612.2	1000	46.7973	953.203	77.85	0.0468
PB600-2-u	608.7	1000	110.746	889.254	73.05	0.11075
PB600-3-u	602.4	1000	22.2017	977.798	81.16	0.0222

Figure B. 7: Liquid Sample Data Sheet for No Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Mushroom Sorption Data Sheet Continued
--Lead (No Pretreatment)--

Sample #	Mushroom Biomass (mg)	[C]o (ppm)	[C]f (ppm)	Ce (ppm)	qe (mg/g)
PB700-1-u	712.6	1000	100.908	899.092	63.09	
PB700-2-u	712.4	1000	56.6355	943.364	66.21	
PB700-3-u	707.3	1000	52.9462	947.054	66.95	
PB800-1-u	816.9	1000	75.0822	924.918	56.61	
PB800-2-u	802.7	1000	81.2311	918.769	57.23	
PB800-3-u	810.2	1000	56.6355	943.364	58.22	
PB900-1-u	905.9	1000	56.6355	943.364	52.07	
PB900-2-u	905	1000	91.0694	908.931	50.22	
PB900-3-u	903	1000	36.959	963.041	53.32	
PB1000-1-u	1032.5	1000	75.0822	924.918	44.79	
PB1000-2-u	1009.6	1000	100.908	899.092	44.53	
PB1000-3-u	1021.2	1000	137.801	862.199	42.21	
C1-H2O+M1000	--					
C2-PB	--					
C3-M1000	--					

Figure B. 8: Liquid Sample Data Sheet for No Pretreatment Method Experiment

Appendix B-4: Lower Concentration with Updated Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Names: William, Danielle, Yue, and Nolan
Location: NAU EGR 245

Mushroom Sorption Data Sheet
--Lead (N_Pre-Liquid-Low 400 ppm)--

Sample #	Mushroom Biomass (mg)	$[C]_o$ (ppm)	$[C]_f$ (ppm)	C_e (ppm)	q_e (mg/g)
PB100-1	103.6	400	260.84	139.16	67.16
PB100-2	109.1	400	100.67	299.33	137.18
PB100-3	101.6	400	136.55	263.45	129.65
PB300-1	307.4	400	108.36	291.64	47.44
PB300-2	304.1	400	135.27	264.73	43.53
PB300-3	305.9	400	201.90	198.10	32.38
PB500-1	501.7	400	127.58	272.42	27.15
PB500-2	503.0	400	160.89	239.11	23.77
PB500-3	499.5	400	137.83	262.17	26.24
PB600-1	607.3	400	100.67	299.33	24.64
PB600-2	601.4	400	68.64	331.36	27.55
PB600-3	602.2	400	64.79	335.21	27.83
PB700-1	699.3	400	76.33	323.67	23.14
PB700-2	702.3	400	107.08	292.92	20.85
PB700-3	700.3	400	77.61	322.39	23.02
PB800-1	802.4	400	51.98	348.02	21.69
PB800-2	801.1	400	44.29	355.71	22.20
PB800-3	799.4	400	46.86	353.14	22.09

Figure B. 9: Liquid Sample Data Sheet for Lower Concentration with Updated Pretreatment Method Experiment

CENE 486 Capstone Project—Adsorption of Copper and Lead from Mine Waste Water Using Mushrooms as a Biosorbent

Environmental W.W. Engineering

Mushroom Sorption Data Sheet Continued
--Lead (N_Pre-Liquid-Low 400 ppm)--

Sample #	room Biomass	$[C]_o$ (ppm)	$[C]_f$ (ppm)	C_e (ppm)	q_e (mg/g)
PB1000-1	1000.4	400	63.51	336.49	16.82
PB1000-2	1001.5	400	46.86	353.14	17.63
PB1000-3	999.5	400	46.86	353.14	17.67
C1-H2O+M1000	1001.5				
C2-PB	--				
C3-M1000	1003.2				

Figure B. 10: Liquid Sample Data Sheet for Lower Concentration with Updated Pretreatment Method Experiment

Appendix C: Percent Removal Raw Data

Table C. 1: Percent Removal Data - Original Pretreatment

Percent Removal for Original Pretreatment Method - 1000 ppm of lead			
Mushroom (mg)	Removal (%)	Mushroom (mg)	Removal (%)
111.7	49.8	700.9	82.5
112.8	52.4	703.8	85.6
105.4	57	705.2	82.3
200	75.7	802.1	82
207.9	64.6	804.3	76
199.8	67.7	801.4	72.2
300.9	67.5	904.3	83
306.4	88.7	902.1	84.4
302.2	83.8	901.5	89.8
406.4	73.5	1000.6	84.4
406.2	82.7	1005.5	87.7
405.8	81.1	1000.3	93.1
504.7	81.5		
505.8	81.1		
502.4	85.1		
609.9	91.4		
608.8	81.6		
602.3	74.6		
Average % Removal=		78.09333333	

Table C. 2: Percent Removal Data - Modified Pretreatment – 1000 ppm

Percent Removal for Modified Pretreatment - 1000 ppm of lead			
Mushroom (mg)	Removal (%)	Mushroom (mg)	Removal (%)
104.6	52.9	705.3	80.1
116.4	59.4	712.3	80.1
113.1	53.5	700.7	78.7
206	70.6	817.9	80.9
218.9	65.4	823.4	62.6
214.6	81.6	813.5	47.3
327.9	84.8	920.8	73.4
320.3	55.9	908.5	83.5
321.5	72.8	906.3	72.5
412.1	76.8	1044.8	74.8
402.1	46.6	1016	80.9
418.7	82.2	1016.5	87.8
519	85.0		
518.1	80.7		
518.9	74.5		
617.5	79.2		
610.7	80.1		
608.3	76.5		
Average % Removal=		72.69730655	

Table C. 3: Percent Removal Data - No Pretreatment

Percent Removal for No Pretreatment -1000 ppm of lead			
Mushroom (mg)	Removal (%)	Mushroom (mg)	Removal (%)
109.3	54.7	712.6	89.9
107	52.4	712.4	94.3
100.6	49.4	707.3	94.7
207.2	71.6	816.9	92.5
213.8	84.6	802.7	91.9
210.6	95.7	810.2	94.3
304.9	97.5	905.9	94.3
308.7	99.0	905	90.9
301.1	80.3	903	96.3
405.1	95.4	1032.5	92.5
403.6	99.1	1009.6	89.9
400.1	96.1	1021.2	86.2
510.5	96.7		
501.8	96.2		
505.4	94.6		
612.2	95.3		
608.7	88.9		
602.4	97.8		
Average % Removal=		88.44169844	

Table C. 4: Percent Removal Data - Modified Pretreatment – 400 ppm

Percent Removal for Modified Pretreatment - 400 ppm of lead			
Mushroom (mg)	Removal (%)	Mushroom (mg)	Removal (%)
103.6	34.8	1000.4	84.1
109.1	74.8	1001.5	88.3
101.6	65.9	999.5	88.3
307.4	72.9		
304.1	66.2		
305.9	49.5		
501.7	68.1		
503	59.8		
499.5	65.5		
607.3	74.8		
601.4	82.8		
602.2	83.8		
699.3	80.9		
702.3	73.2		
700.3	80.6		
802.4	87.0		
801.1	88.9		
799.4	88.3		
Average % Removal=		74.222098	

Table C. 5: Percent Removal Data - Modified Pretreatment - 400 and 1000 ppm

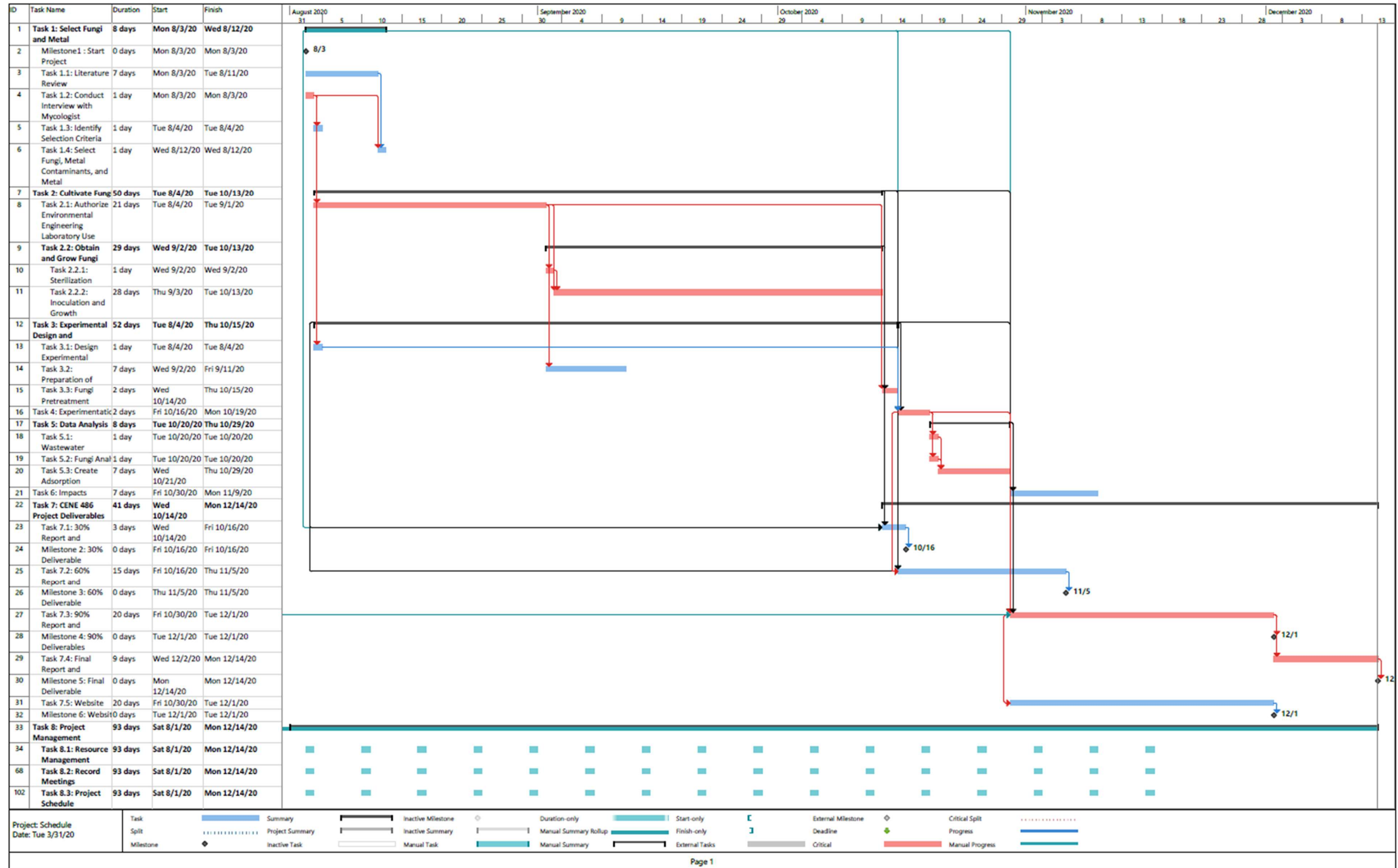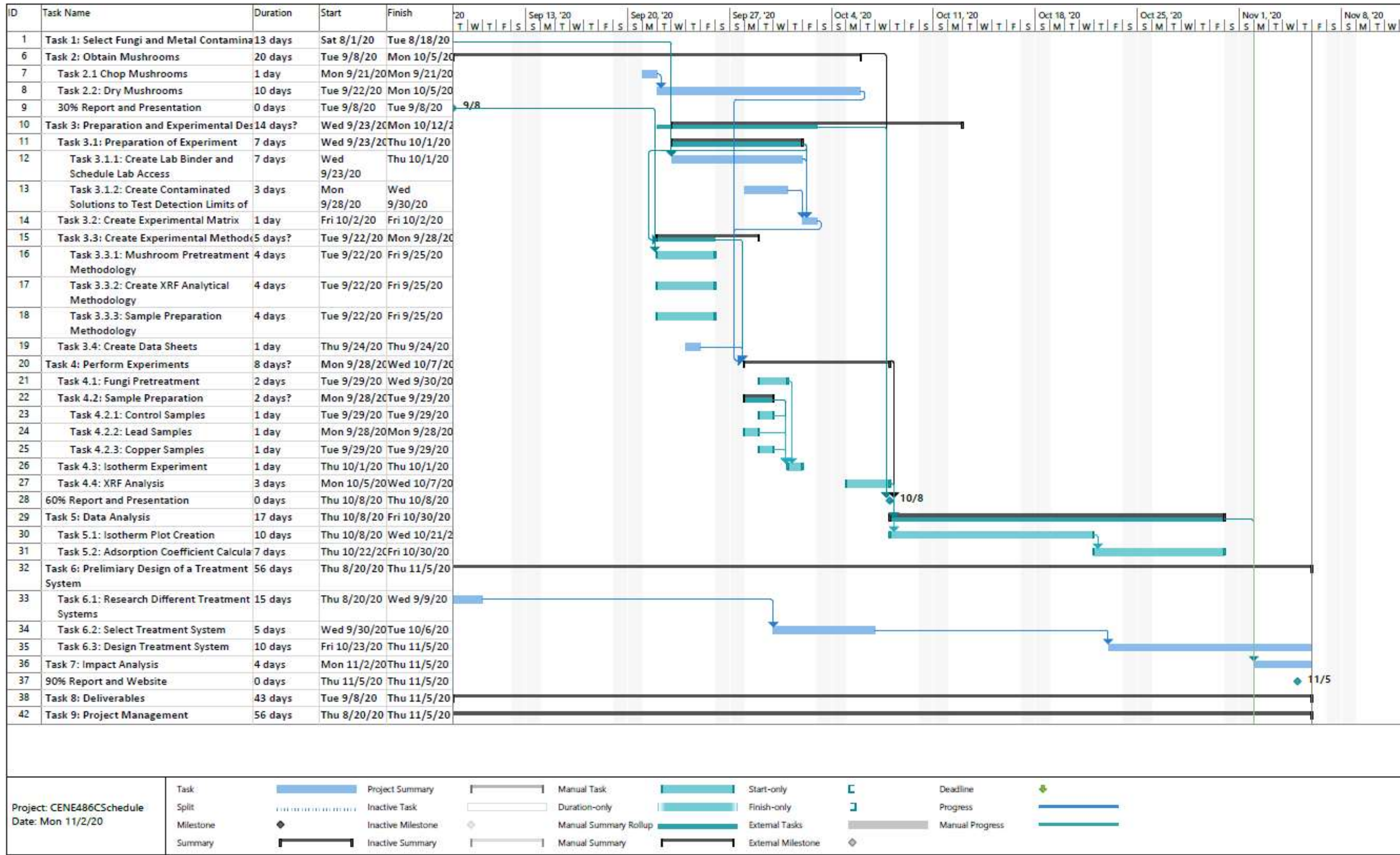| Percent Removal for New Pretreatment - 400 and 1000 ppm of lead							
1000 ppm Starting Concentration				400 ppm Experiment Starting Concentration			
Mushrooms (mg)	Removal (%)	Mushrooms (mg)	Removal (%)	Mushrooms (mg)	Removal (%)	Mushrooms (mg)	Removal (%)
104.6	52.9	705.3	80.1	103.6	34.8	1000.4	84.1
116.4	59.4	712.3	80.1	109.1	74.8	1001.5	88.3
113.1	53.5	700.7	78.7	101.6	65.9	999.5	88.3
206	70.6	817.9	80.9	307.4	72.9		
218.9	65.4	823.4	62.6	304.1	66.2		
214.6	81.6	813.5	47.3	305.9	49.5		
327.9	84.8	920.8	73.4	501.7	68.1		
320.3	55.9	908.5	83.5	503	59.8		
321.5	72.8	906.3	72.5	499.5	65.5	Average % Removal for 400 ppm experiment = 74.2 %	
412.1	76.8	1044.8	74.8	607.3	74.8		
402.1	46.6	1016	80.9	601.4	82.8		
418.7	82.2	1016.5	87.8	602.2	83.8		
519	85.0	Average % Removal for 1000 ppm experiment = 72.7 %		699.3	80.9		
518.1	80.7			702.3	73.2		
518.9	74.5			700.3	80.6		
617.5	79.2			802.4	87.0		
610.7	80.1			801.1	88.9		
608.3	76.5			799.4	88.3		
Overall Average Percent Removal = 73.5%							

Appendix D: Gantt Charts

Figure D. 1: Proposed Gantt Chart

Figure D. 2: Actual Gantt Chart