Moss Revegetation, A Process to Initiate Restoration and Repair of Natural Resources Damaged by Mining that Impact Butte Area One

A Proposal to the Montana BNRC/NRDP

October 16, 2015

Dr. Grant G. Mitman
Research Professor
Montana Tech of The University of Montana
1300 West Park Street, Butte MT 59701
gmitman2@mtech.edu
406-496-4617, 406-490-3177 (cell)

Mielichhoferia mielichhoferiana (Funck) Loeske Mielichhof's copper moss revegetation growing on mine waste tailings at Badger State Mine-Butte Montana (Mitman research 2003)

Commercial Moss Revegetation RST Environmental http://www.rst.co.nz/

Moss Revegetation, A Process to Initiate Restoration and Repair of Natural Resources Damaged by Mining that Impact Butte Area One

A. Contact Information

Dr. Grant G. Mitman
Research Professor
Montana Tech of The University of Montana
1300 West Park Street, Butte MT 59701
gmitman2@mtech.edu
406-496-4617, 406-490-3177 (cell)

B.1. Project Summary and Map

Mosses are plants found in the Kingdom: Plantae, Phylum (Division): Bryophyta; they reproduce by spores or plant fragments; lacking roots or vascular tissue they must draw water from the air or soil directly into their "leaves." Mosses are well known as pioneer plants; they the very first plants to establish themselves in disturbed soils and barren areas. The establishment of mosses and their associated organisms accelerate the natural processes that restore damaged areas transforming them into normal, steady state, biodiverse ecosystems. Other researchers have proved that mosses can establish stable biomats that are the ideal system for initiating restoration of mine waste sites (Stanley et al 2000, Coulson et al 2005). These extremophiles have tremendous potential for the initiation of natural revegetation of mine waste areas. First, mosses do not require good quality soil; they produce it. In addition, the rehabilitation of mine dumps or disturbed areas by native or nonnative vegetation is usually a very slow or even a nonexistant natural process. The removal of mine waste, capping and replacement of soil, and subsequent replanting of the affected areas is usually necessary. This type of restoration is an expensive project, and when a responsible party cannot be identified and thus the sites remain barren. Consequently, rains and wind continuously transport the contaminants from the overburden into and the aquifer. Moss communities and their associated organisms will slow or prevent this process. They create rich soil by building up organic material from their growth and by trapping silt and soil from the environment (wind and rain). Moss community organisms also fix atmospheric nitrogen into bioavailable nitrogen. Finally, Moss communities are self maintaining without supplemental irrigation. These plants are not only resistant to metals and metalloids, but they also adsorb these toxic substances and remove them from the surrounding soil. In this way, mosses provide a foundation for a natural succession of other plant communities to build a stable ecosystem.

This grant proposes a specific research goal. The practical application is to identify hardy moss species from mine waste areas, grow them into propagation beds, and subsequently introduce them back into the disturbed areas to initiate restoration. A variety of hydrophilic polymers and other soil amendments will be applied to sites to improve the establishment and growth rates of moss. This data will provide an understanding of how researchers can initiate repair and restoration of mine waste environments by use of native mosses. This proposal requests $48, 979 for a PI and Graduate Student to study and restore mine waste damaged ecosystems from extreme to mildly contaminated soil areas affecting the BAO (Butte Area One) watershed. Funding of this proposal will be a significant opportunity to invest in the future of restoration and repair of natural resources by investigating the revegetation potential of these extremophile plants.

B.2. Background

Historical records document that Mosses have been known for their ability to grow on sites rich in metals where nothing else would grow for more than 5.000 years. In fact, a certain group of mosses known as the "Copper Mosses" were mined for their copper content and used to find the best sites to dig for ore. *Mielichhoferia mielichhoferiana* is one of the copper mosses that is common throughout the Butte Area. Another advantage of mosses is that they are easily propagated. To cover a bare area of soil with moss, it is sometimes as easy as collecting some plant material, breaking it apart in a commercial blender, allowing the fragments to grow into colonies and then finally transferring them to the soil. While this technique works quite well in wet environments, it does not work as well in dry areas like Montana. Fortunately, horticulturists have developed a solution to re-seed dry soils, hydrophillic polymers and starch compounds; these polymers work equally well for moss growth. Mosses can sequester and stabilize staggering amounts of metals from soil (Table 1). Experiments (Henderson MS thesis 2005), show metal concentration factors in moss from Badger State Mine Soil from Butte (Table 2) More important, mosses can help establish microbial mats; many scientists consider these mats to be the best restoration technology available for soils and for the initiation of stable, plant community succession.

Table 1. Metal Concentrations in Selected Mosses (Vymazal 1995)

Rhynchostegium riparoides	concentration of metal mg/kg dry mass
Fe	1,810-143,000
Mn	280-143,000
Mg	756-3,730
Zn	128-22,300
Mo	0.23-0.79
Cd	0.50-89.5
Cr	2.3-1,330
Pb	30.9-17,800
Fontinalis sp.	
Cu	10-1,900
Ni	3-1,300
Scapania undulata	
As	0-2,190
Jungermania vulcanicola	40-13,500

Table 2. Metal concentration Factors in Moss as a Ratio of those in the Badger State Mine Soil after 8 weeks. (Henderson 2005)

	Mg	Al	Si	Mn	Fe	Ni	Cu	Zn	Cd	Pb
ppm in soil	8,893.4	24,721.8	464.9	1,005.1	21,121.3	10.9	306.9	764.3	4.1	171.4
conc in moss	0.65	0.49	2.20	1.23	0.21	1.65	4.27	3.61	9.78	0.14
ppm in moss	5,668.4	14,511.3	887.1	1,008.3	4,735.0	18.0	1,350.5	2,717.8	31.8	22.4

Butte, Montana is an area rich in mine waste sites that need revegetation, restoration and repair of its natural resources. It is easy to find hundreds of sites characterized by years of mining, milling, and smelting waste to test the moss restoration processes. Thus, the research potential for this restoration approach is tremendous. Nevertheless, the only solutions to restoration are often chemical in nature or involve physical removal of soils, and researchers' often overlook biological aspects. When they restore a site, implementation of the species composition of these sites is critical, because not all organisms will grow at any particular site. Thus, it is critically important that organisms used to restore the specific site in are suited to the specific substrates in question. If you build it, they won't necessarily come; one may have to construct the species composition to fit the specific revegetation task! This is why a moss/microbial mat is the ideal solution for revegetation of mine waste; it can be constructed to suit the specific site.

Microbial Moss mats are not a new idea. Environmental restoration companies are currently implementing them in many parts of the world to restore mine waste and overburden rock dumps (Stanley et al. 2000) to remove copper, iron, manganese, zinc, silver, chromium, cadmium, lead, nickel and a variety of other metals and even toxic organic compounds to initiate revegetation (Phillips and Bender 1998).

Advantages of Moss Mats for restoration of mine waste sites:

- Mosses are pioneer plants that are normally the first to grow on disturbed sites
- Mosses are easily propagated from plant fragments into colonies
- Mosses initiate the recruitment and survival of other plants (During and van Tooren 1990; Sohlberg and Bliss 1984)
- Moss establishment provides an increase in soil moisture
- Mosses transport heir excess sugars and organic carbon from photosynthesis into the soil increasing soil microorganism communities and soil fertility
- Mosses are not consumed by nearly any animal known. Thus, metal accumulation is not a problem for wildlife; they do not require pesticide. application
- Moss microbial mat organisms will buffer each other from environmental disturbance.
- Moss mats increase soil fertility by fixing atmospheric nitrogen to bioavailable forms providing an increase in soil fertility.
- Mosses build soil by trapping sediment,organic materials and by their own decomposition and physiological products and
- Mosses will stabilize the soil surface to prevent contaminated soil from being distributed by wind and rain into the air and the watershed
- Moss mats, once established are self-sustaining and do not require irragation, fertilizer or other soil additives
- Moss mats can be engineered to comprise a species composition that is compatible with the site
- Moss mats are persistent in nature.
- Moss Biofilms raise pH, and increase alkalinity.
- Moss Biofilms are dessication resistant.
- Moss mats have a high resistance to the effects of heavy metals, toxins and harsh environments.
- Mosses are low cost to establish and maintain.

B.3. Maps.

Figure 1. Copper Mountain Recreation Complex-Arial View.

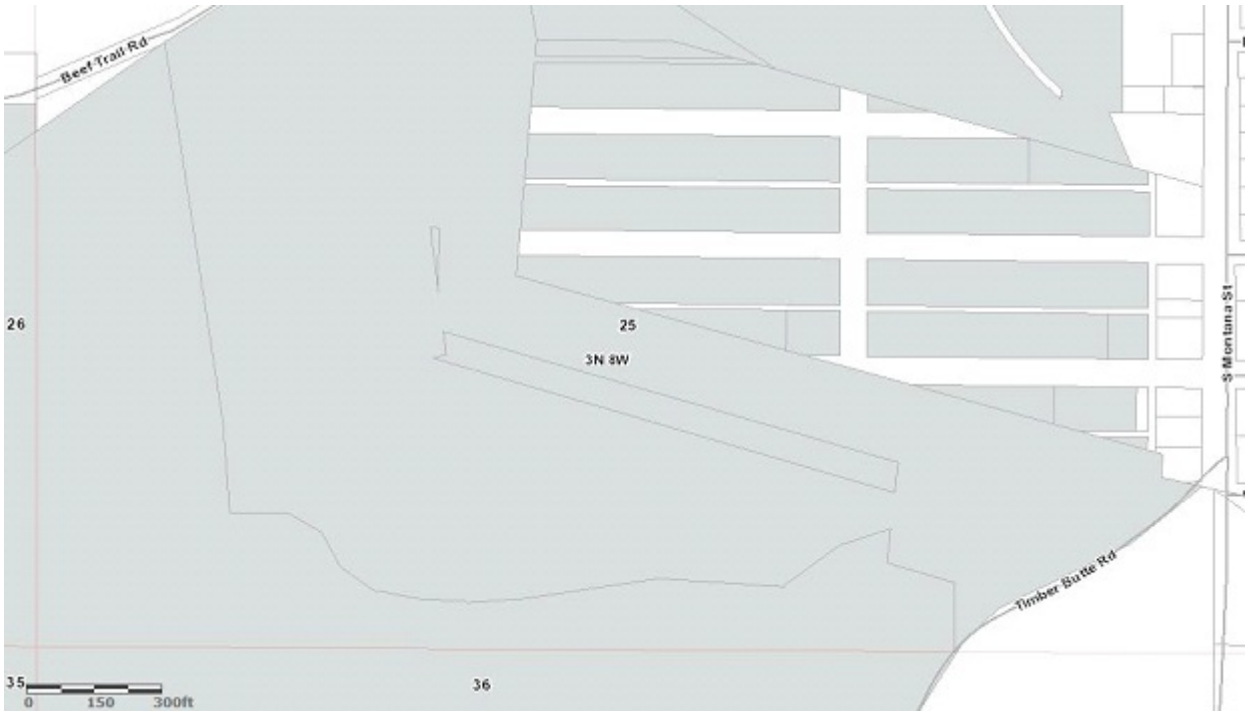

Figure 2. Copper Mountain Recreation Complex-County View.

Figure 3. Copper Mountain Recreation Complex-close up.

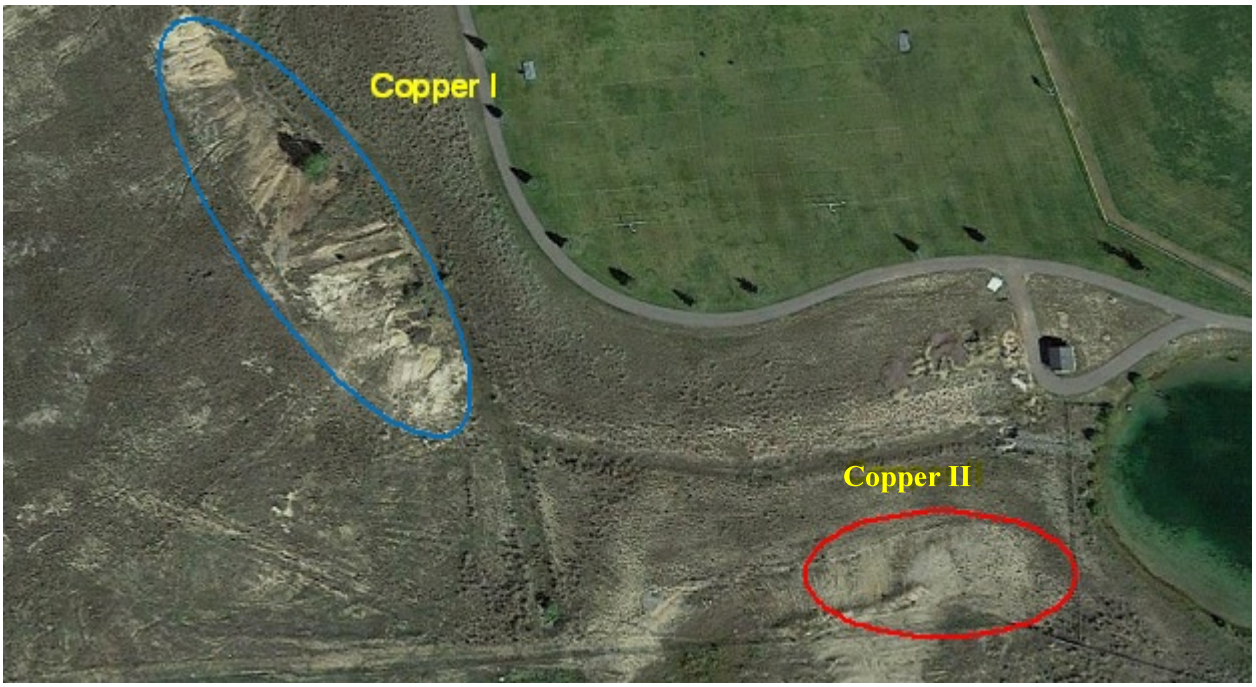

Figure 4. Ryan Mine and Walkerville Drive-Arial View.

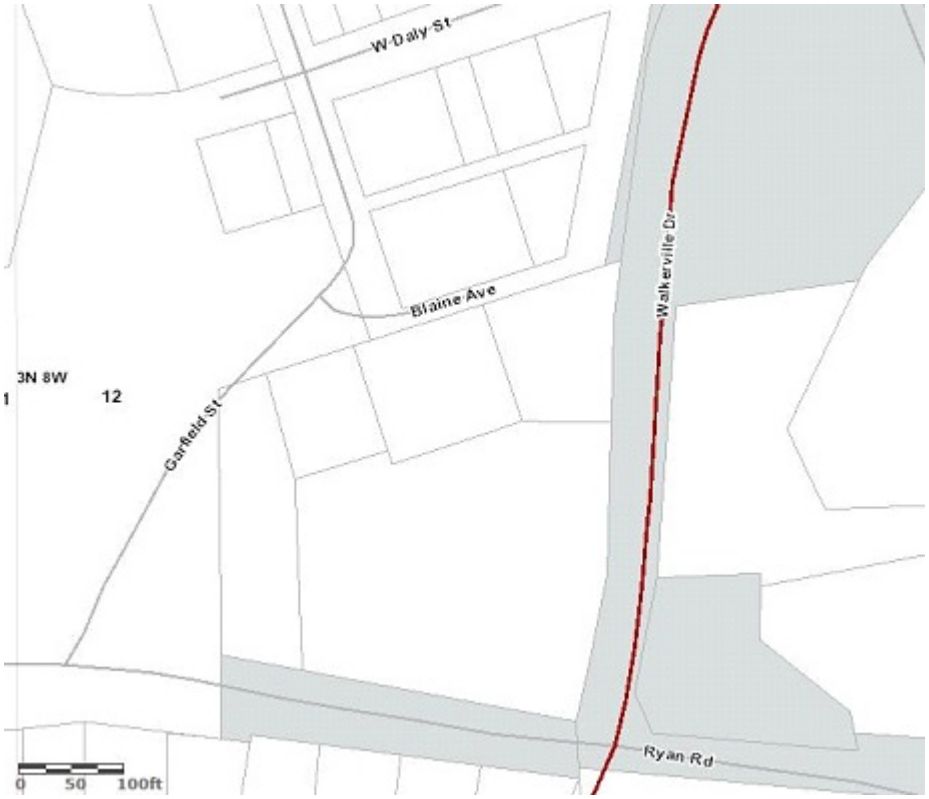

Figure 5. Ryan Mine and Walkerville Drive Road-County View.

Figure 6. Ryan Mine -Closeup.

Figure 7. Walkerville Drive -Closeup.

C. Project Goals and Objectives

The Primary goal of this project is to develop an easily replicated method to revegetate, and restore mine impacted areas not designated for natural resource repair, sites that are cost prohibitive to address, locations for which there is not a responsible party, or overburden that is found on private land. This technology would address natural resource recovery for the many locations affecting BAO for which the expensive removal and replacement or capping and replanting of the affected areas does not occur, for the previously listed reasons. Without revegetation of some kind, the persistent barren mine waste sites ensure the continued erosion and transport of the contaminated overburden into the BAO aquifer.

Secondary goals of this project will involve the development of a moss hydration mixture for mine waste areas consisting of optimizing:

- Three hydrophilic polymer supplements,
- Osmocote (slow release fertilizer)

Along with these physical variables different moss species will be identified, characterized, and tested with respect to their ability to revegetate different types of disturbed sites. The Mitman Lab will maintain these moss cultures along with detailed descriptions of the soil type and moss hydration mixtures for future use.

D. Project Benefits

To summarize, the project benefits or this research includes (Details see page 4):

1. Revegetation, Restoration and Repair of mine waste affected areas
2. Provide a suitable substrate for the initiation of stable vascular plant communities
3. Development of an easy and cost-effective protocol to revegetate mine waste sites
4. Provide a soil water retention ecosystem that will reduce transport of contaminated water runoff and wind blown soil into the BAO aquifer
5. This Project represents a prototype of a low cost, easily reproducible method to revegetate barren mine waste and other areas
6. Information obtained from this project will be used to construct a poster for display purposes for various venues
7. Publish step by step "how to" pamphlet so that individuals and other agencies may continue to use this application technology

It is important that this technology be easily transferable to anyone who wishes to implement it, and not be buried in a scientific publication.

E. Project Implementation

E.1 Moss Collection

The first step will be to collect, identify and isolate different moss communities/species from mine waste sites and associate them with each soil type so that the procedure for revegetation may be replicated. It is important that care and effort will be taken with this sample collection (eco-sourced) so that sites are not damaged. In other words, only small representative samples will be collected, not just scoop up everything in sight. Mosses are easily grown and propagated from very small plants or plant fragments, so collection of massive amounts of material is not necessary.

E.2. Propagation

A single cell of a moss plant can grow into an entire plant given time (blender technique). However, in order to speed up the regeneration process, mosses will be propagated into mats by using either fragments or by applying small clumps of moss to trays with acidified commercial soil. The method selected for developing propagation mats is species dependent and will be determined during the course of this research. Mosses will be grown in standard 1020 Greenhouse trays (11" W x 21.37" L x 2.44" D) with acidified commercial soil in a dedicated temporary greenhouse (Rhino PEAK - 22'W X 24'L X 12'H) so as not to interfere with other ongoing projects at Montana Tech (discussion with Dr. Robert Pal). During this growth process techniques for rapid moss propagation will be developed so that large scale application and implementation methods will be possible in the future. This process will be continuous since moss will be needed for the duration of the revegation study.

E.3. Soil Moist Testing

Three different products produced by JRM Chemical, Inc will be tested in Greenhouse studies and in the field prior to application of mosses to sites in a matrix to determine the optimal combination and application rates. Although this study is already underway for roadcuts, it needs to assessed for specific species of mosses and soil types. Table 3.

The three products that will be used are:
1. Hydrobond™ which is a safe, non-toxic linear high molecular weight anionic polymer which acts as a tackifier to keep the soil moist without drawing moisture out of the plant
2. Soil Moist Fines which is a safe, non-toxic synthetic crosslinked, acrylic polyacrylamide with a potassium salt base (50-500υm).
3. Soil Moist Natural is a grafted starch polymer derived from corn designed to reduce plant watering. It can hold several hundred times its weight in water which it releases to the plant as the soil dries out.

Table 3. Treatment Matrix

Hydrobond,	Fines	Natural
Hydrobond+Fines	Fines+Natural	Natural+Hydrobond
Hydrobond+Fines+Natural	Osmocote only	no treatment

Soil will be fertilized by Osmocote Classic, an extended release (14-16 month) fertilizer19-6-12 (Nitrogen-Phosphorous-Potassium) at a rate of 25g/ft$^{2.}$.

E.4. Soil Analysis

Soils from each site will be characterized for metals by the University of Georgia Laboratory for Environmental Analysis (LEA) so that for future work specific mosses may be associated with metal concentration for continued revegetation projects. pH and other physical soil parameters will also be collected. Soils from the four sites will also be characterized by identifying species and percent cover of mosses and plants already present.

E.5. Application.

E.5.1. Matrix Trials

Application will begin as soon as moss can be propagated to regenerate sufficient quantity in the greenhouse beds. Each of the four (4) sites (see maps B.3., Figures 1-7, pages 5-8) will be tested for the effects of Soil Moist by using a matrix (seven (7) combinations) with and without moss application as delineated in E.3. , one site with only Osmocote and one site with no treatment. This will determine a baseline control and also to determine if there are any beneficial effects of the Soil Moist or Osmocote products to the soil without moss. Replicates of three (3) for each of the eighteen (18) treatments will be applied in 0.25m^2 quadrants. Moss will be applied to the sites by the Cookie sheet method (Martin 2015). This method has had good results in field trials and involves placing small colonies of moss across the prepared sites, watering them and then pressing clumps into the soil. Implementation of Matrix trials will occur in Spring 2016 (if possible).

The results of this Matrix trial will yield sufficient data to determine the optimal application of soil moist and moss to treat the complete area the four different mine waste areas. As soon as this data is analyzed, the remainder of that area will be treated with that optimal treatment.

Table 3. Treatment matrix.

Hydrobond,	Fines	Natural
Hydrobond+Fines	Fines+Natural	Natural+Hydrobond
Hydrobond+Fines+Natural	Osmocote only	no treatment

E.6. Dissemination of Results

Data, results and their interpretation will be reported to BNRC/NRDP in the form of technical reports. A report and presentation will be available to BNRC each year with a final report submitted spring 2019. In addition, a step by step "how to" pamphlet will be produced on how to make use of the technology of mosses for restoration and repair of natural resources. Finally, a poster will be produce that summarizes our findings and how to implement Moss revegetation. This will be presented to BNRC and made available to any other interested parties on request.

F.1. Project Schedule

The proposed work will begin as soon as funding becomes available and as soon as the supplies can be delivered. The work plan for this three year project will consist of three phases:

Phase I will consist of moss collection, propagation, soil moist testing, soil analysis.

Phase II is application of moss to the damaged sites Spring 2016, Fall 2016 and possibly spring 2017 depending on initial results.

Phase I and II will occur all within the first year of the project, monitoring will be over all three years.

Phase III will consist of monitoring beginning in year one (2016) and continue until spring 2019. Initial monitoring will be conducted every 2 weeks the first year and monthly the second 2 years. (see G.1.Monitoring Activities page 12).

A report and presentation will be available to BNRC/NRDP each year with a final report submitted spring 2019.

G. Monitoring Activities

Initial monitoring of quandrats for the four sites studied will be conducted every 2 weeks the first year and monthly the second 2 years. After application to the complete area of the four sites, data will be sampled by observing random quadrants along a transect line. In order to determine project effectiveness, the increase in percent cover of moss and other plants will be assessed in terms of increases in both species diversity and plant growth percent cover and explained by biostatistical analysis.

H.1. Project Budget

BNRC Budget						
Mitman 10/9/15						
		Monthly	# of		# of	Cost
A.	SALARIES AND WAGES	Rate	Months	BNRC	Months	Share
	Grant Mitman	$7,682	2	$15,364	2	$15,364
	Graduate Student - Academic Year	$1,200	2	$2,400		
	Graduate Student - Summer	$2,400	2	$4,800		
	Subtotal			**$22,564**		**$15,364**
B.	FRINGE BENEFITS					
	46% Contract Professionals			$7,067		$7,067
	3% of Students/Academic Year			$72		
	10% Students/Summer			$480		
	Subtotal			**$7,619**		**$7,067**
C.	OTHER DIRECT COSTS					
	Supplies			$8,000		
	Travel			1,000		
	Subtotal			**$9,000**		**$0**
D.	PARTICIPANT SUPPORT COSTS					
	Graduate Student Fee Waiver ($5,800/yr)			$0		$5,800
	Subtotal			**$0**		**$5,800**
E.	TOTAL DIRECT COSTS			**$39,183**		**$28,231**
F.	INDIRECT COSTS (25% of TDC)			**$9,796**		**$7,058**
G.	**TOTAL PROJECT COST**			**$48,979**		**$35,289**

H.2. JUSTIFICATION OF BUDGET

The research supples requested for this proposal covers the goals for this research to evaluate the isolated mine waste moss species for use of an experimental matrix for revegetation. Two months salary are requested as salary for Grant Mitman (PI) from BNRC/NRDP and will matched by 2 months time (1:1) as a match by Montana Tech at my academic salary rate. Two months salary Salary and benefits are explained in the budget Table. Graduate student support of 4 months is requested from BNRC/NDRP for graduate student support, two during the academic year and 2 during summer (see budget detail).Montana Tech will also supply a graduate student fee waiver of $5,800. As a result Montana Tech is matching this proposal at 42.%.

The largest cost item will be a large greenhouse from rhino shelter http://www.rhinoshelters.com/greenhouses/40-greenhouse-peaked.html $2,050.00. Other supplies are Detailed below. Miscellaneous supplies will account for consumables, shipping of supplies required for the project.

Detail Budget Table

Greenhouse	$2050.00
Osmocote	$300.00
Soil Moist	$1,000.00
Soil/moss analysis LEA labs	$1,000.00
Kelway pH/moisture meter (2)	$500.00
Soil	$300.00
Moss trays	$1000.00
Misc supplies	$1,850.00
Total	$8,000.00

I. References.

Buxton, R., Stanley, P., Alspach, C., Morgan, and Ross, C. Mosses and Other Early colonizers: Pioneers for Revegetation Mine waste Rock Dumps and Rock Walls. National Meeting of the American society of Mining and Reclamation . Breckenridge, CO.

Coulson, R., Stanley, J. Buxton, R., Ross, C, Alspach. P and Morgan, C. 2005 Hydroseeding indigenous Mosses and Seeds for Successful Rehabilitation..

Henderson, M. 2005. Stabilization of Mine Waste Rock Using Moss. MS thesis Montana Tech.

Martin, A. 2015 Moss Gardening. Timber Press.