A. CONTACT INFORMATION

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B. PROJECT SUMMARY AND MAP

Title: ***"Microbial Activity in Silver Bow and Blacktail Creeks"***

Sponsor: The Laboratory Exploring Geobiochemical Engineering and Natural Dynamics (LEGEND) is the research group headed by Assistant Professor Alysia Cox in the Department of Chemistry and Geochemistry at Montana Tech. The lab consists of five MS in Geochemistry students and three undergraduate researchers. Dr. Cox has a BS in Geological Sciences from the Barrett Honors College and Arizona State University and a PhD in Chemical Oceanography from the Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution with extensive field research and data integration experience. The LEGEND Masters students have strong backgrounds in chemistry, geology, biology and interests in remediation. The LEGEND undergraduate students are majoring in Environmental Chemistry and Biochemistry and have a combined five semesters and two summers of research experience in with LEGEND. Undergraduate Jordan Foster has received the Best Poster Department of Chemistry and Geochemistry and the Laurie Henneman Outstanding Student Presentation Award for Best Undergraduate Student Oral Presentation for his poster at Techxpo and talk at the Montana Academy of Sciences in April of 2016, both entitled "Stormwater in Silver Bow and Blacktail Creeks: Implications for the microbial community".

Project focus: This project will produce the first view of microbial diversity and activity in the Butte Area One and integrate corresponding geochemical data in the area. Results will be compared to less impacted Upper Blacktail Creek. Results will also be compared to the Upper Clark Fork (current funded project of the PI by the Montana Water Center entitled

"Characterizing Microbial Activity as Related to Water Quality in the Clark Fork Headwaters: A Baseline Study").

Partners and implementation:

Project location: The microbial identification and activity sampling will occur at five sites along Silver Bow Creek and Blacktail Creek in the Butte Area One with analyses being performed at Montana Tech. A sixth site on upper Blacktail Creek will serve as a control.

Timeline: The project will begin in May 2017 when funding is expected to become available. LEGEND has already begun sampling for this project starting in November of 2015 (sampling campaigns in Nov 2015, Feb 2016, May 2016, August 2016, November 2016 have been completed); geochemical analyses have been completed for four of these sampling collection days and microbial analyses are currently underway. Please see the following table for the project timeline. Continued sampling for microbial identification and activity will proceed in late May 2017 as classes end and LEGEND students are available full time for field work and analyses. Analyses will occur concurrently and carry on into the fall 2017 and into 2018. A final report will be completed by April 2019 and a final presentation to the BNRC will be made at that time.

Map: Five Butte Area One sites and a control site on Upper Blacktail Creek where microbial identification and activity will be determined at four different times of year (spring baseline, during stormflow, after the stormflow and summer baseline) over multiple years (Figure 1). Sites were chosen in consultation with Joe Griffin.

Figure 1: Map of Butte Area One showing sampling locations.

	Project Timeline																							
	2017								2018												2019			
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Microbial Identification and Activity with Geochemistry																								
Sampling	X			X			X			X			X			X			X			X		
Analysis		X	X	X	X	X		X	X		X	X		X	X		X	X		X	X		X	
Tech Geochemistry MS Thesis													X											
Final Report and Presentation																					X	X	X	X

C. Project Goals and Objectives:

This project will achieve the goals of 1) providing much-needed microbial identification and activity information to the BNRC and Butte community in order to help make informed decisions about Butte Area One restoration and 2) integrate corresponding geochemical data with microbial results and give a holistic view of the watershed encompassing Butte Area One.

Project implementation will occur over a two-year period while accomplishing the following objectives:

1. Determine microbial community structure (identification) in the sediments and waters of Silver Bow and Blacktail Creeks.
2. Determine microbial activities (what reactions are happening) in the sediment and waters of Silver Bow and Blacktail Creeks.
3. Integrate microbial identifications and activities with concurrently collected geochemical data.

D. Project Benefits:

This project aims to determine the microbial community structure and microbial activities in the sediments and waters of Silver Bow and Blacktail Creeks in order link microbial processes with the geochemistry of restoration so that decisions made in the future will be informed ones. This will be achieved by sharing results in a technical report and presentation to the BNRC, a MS thesis in Geochemistry at Tech, and a published manuscript in a journal such as Environmental Science and Technology or Frontiers in Microbiology. This project will yield new information about microbial identity and activity in Silver Bow and Blacktail Creeks that we will link to the geochemistry of restoration with changing seasons and degree of restoration.

E. Project Implementation:

Staff: The following individuals are the members of **LEGEND (Laboratory Exploring Geobiochemical Engineering and Natural Dynamics)** who will be most involved in this project, with the exception of environmental expert, Joe Griffin. Each will contribute to this project, detailed under 'Tasks'. Specific duties are listed below:

- **Dr. Alysia Cox**, assistant professor of Environmental Chemistry at Montana Tech, as the principal investigator and head of LEGEND, will organize weekly lab meetings to keep the project on track, will provide planning oversight and expertise, compose quarterly reports, prepare financial documentation, and be responsible for the completion of the published manuscript, final technical report, and presentation to the BNRC.
- **James Foltz**, LEGEND MS Geochemistry student, has a background in geology, biology, and chemistry and a keen interest in restoration. He will help orchestrate field sampling, be responsible for DNA extractions and analyses, contribute to the published manuscript, and part of his thesis will be related to this work.

- **Shanna Law**, LEGEND MS Geochemistry student, has a background in geology and chemistry. She will help orchestrate field sampling, be responsible for protein extractions, and contribute to the published manuscript.
- **Jordan Foster**, LEGEND Chemistry and Biology undergraduate student, will be involved in field sampling and microbial identification analyses. He will help compose text and figures for the various publications.
- **Joe Griffin**, local environmental and water chemistry expert and consultant, will advise and consult on the project.

Joe Griffin is willing to provide preexisting data for this project and contribute 80 hours of expertise to the planning and execution of the project (see attached letter). All microbial identification (DNA) and activity (protein) extractions will be performed by LEGEND at Montana Tech lab. DNA for sequencing will be sent to MRDNA (Shallowater, TX). Protein analyses will now be performed in house in the Chemistry and Geochemistry Department at Montana Tech. Concurrent water samples will be analyzed at the Montana Bureau of Mines and Geology. Collaboration with Dr. Chris Gammons on his project will be orchestrated pending funding of both projects. We would perform microbial analyses coordinated with his sampling.

Tasks: Individual tasks required to complete this project will be undertaken according to the previously listed objectives. Specific tasks vital to each objective are listed below:

- **Objective #1** - execute our sampling plan taking advantage of our consultations and previous experience as well as storm flow events. All LEGEND members participate in sampling expeditions. Samples will be extracted for DNA (microbial identification) by James Foltz and Jordan Foster and sent to MRDNA (Shallowater, TX) for sequencing. Water samples will be analyzed at the Bureau.
- **Objective #2** - extract protein (microbial activity) in the lab by Shanna Law and analyze in house.
- **Objective #3** - integrate microbial data with corresponding geochemical analyses and existing data. All involved will work on analyzing and plotting data to determine correspondence and trends.

Preliminary product descriptions:

Tech MS thesis - One of the MS in Geochemistry students will use the first part of this project as the focus of their thesis at Tech, to be completed in May 2018.

Published Manuscript - This will be based on the MS thesis and will be published at the end of the project.

Technical Report - This report will be a summary of the work and given to the BNRC.

Technical Presentation - This presentation will be given to the BNRC at the end of the two year project.

F. Project Schedule:

The project will begin in May 2017 with the onset of funding. We already have geochemical data from the first set 4 sample collections and microbial analyses are underway. We will collect samples in May 2017. The students will be available for summer research and any leftover microbial analyses from the first seven collections will be completed during the summer. Analyses will be performed during the summer and into fall. After each sampling event (every three months), water chemical and microbial analyses will be performed and analyzed.

Quarterly reports will be submitted on time and in coordination with achievement of objectives described as follows: Objectives #1and #2 will be partially completed in August 2017, with more information after each subsequent sampling event. Objective #3 will be achieved by January of 2018, with more information after each subsequent sampling event. A final report will be submitted in April 2019.

G. Monitoring Activities:

Progress and effectiveness will be measured by filling out checklists for the completion of the tasks required to complete each objective. These checklists are an inherent part of the project progression and will be completed and turned in with the quarterly reports.

H. Project Budget

The budget narrative below shows the details and calculations for the budget. Montana Tech's budget summary follows. In-kind match is provided through Tech by Dr. Cox's time during the academic year, which includes benefits and indirect costs. Some samples have already been collected and analyzed that will contribute to the products produced by this project. The analysis costs are included as match. Also, the Montana Bureau of Mines and Geology Analytical Lab offers a discount for Tech student projects, this is also counted as match (see attached letter). Joe Griffin has also offered his consulting time as match (see attached letter).

Budget Narrative:

Microbial Identification and Activity with Corresponding Geochemistry:

A. SALARIES AND WAGES

Organization, Supervision, and Writing - Dr. Cox, 1 month x $7,645/month = $7,645 requested; Dr. Cox, 3 months x $7,645/month = $22,935 in-kind

Sampling, DNA Extraction, and Analysis - graduate student James Foltz, 2.5 months salary x $2400/month = $6000

Sampling, Protein Extraction, and Analysis - graduate student Shanna Law, 2.5 months salary x $2400/month = $6000

Sampling, Assisting with Extractions, and Analysis - undergraduate Jordan Foster 2.5 months x $1600/month = $4000

Subtotal = $22,056 requested; $18,168 in-kind

B. FRINGE BENEFITS – 25% Faculty, 10% Students Summer

Faculty - $1,911 requested; $5,734 in-kind

Students - $1,600

Subtotal = $3,114 requested; $4,542 in-kind

C. OTHER DIRECT COSTS

Supplies - sampling vials, sampling materials, dry ice or liquid nitrogen, DNA extraction kits, DNA extraction consumables, protein extraction consumables = $4000

Sample Analysis – DNA sent to MRDNA for sequencing (84 samples x $87.50) = $7,350; water brought to the Montana Bureau of Mines and Geology for water chemistry (54 samples x $62.50) = $3,375, water samples already analyzed by the MBMG (36 samples x $125) = $4500 in-kind; discount for samples to be analyzed by the MBMB (54 samples x $62.50) = $3,375 in-kind, all protein samples analyzed in house for $525. Total sample analysis = $18,750 requested; $7,875 in-kind

Publishing Costs - an open access manuscript for $2,500 (Frontiers in Microbiology or Environmental Science and Technology)

Subtotal = $18,750 requested; $7,875 in-kind

D. NA

E. TOTAL COSTS

$50,306 requested; $36,544 in-kind

F. Consultant - Joe Griffin

80 hours x $110/hr = $8,800 in-kind

G. TOTAL PROJECT COST

$50,306 requested; $45,424 in-kind

Grant Total: Amount Requested = $50,306 In-kind Match = $45,424

Budget Summary

BNRC Budget

Alysia Cox November 2016

		Monthly Rate	# of Months	BNRC	# of Months	Cost Share
A.	SALARIES AND WAGES					
	Alysia Cox	$7,645	1	$7,645	3	$22,935
	Graduate Student - Academic Year (20hrs/wk)	$1,200				
	Graduate Students - Summer (40 hrs/wk)	$3,000	5	$15,000		
	Undergraduate Student - Academic Year					
	Undergraduate Students- Summer	$2,000	2.5	$5,000		
	Subtotal			**$27,645**		**$22,935**
B.	FRINGE BENEFITS					
	25% Faculty			$1,911		$5,734
	3% of Students/Academic Year			$0		
	10% Students/Summer			$2,000		
	Subtotal			**$3,911**		**$5,734**
C.	OTHER DIRECT COSTS					
	Supplies			$5,000		
	Contracted Service/Sample Analysis			$11,250		$7,875
	Other/Publishing Costs			2,500		
	Subtotal			**$18,750**		**$7,875**
D.	PARTICPIANT SUPPORT COSTS					
	Graduate Student Fee Waiver ($5,800/yr)			$0		
	Subtotal			**$0**		**$0**
E.	TOTAL COSTS			**$50,306**		**$36,544**
F.	Consultant - Joe Griffin					**$8,880**
G.	**TOTAL PROJECT COST**			**$50,306**		**$45,424**

November 14, 2016

Alysia Cox, PhD

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Montana Tech of the University of Montana

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St. Butte, MT
59701

Office: CBB 217

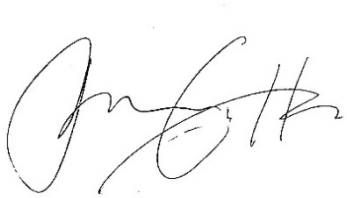
(406) 496-4185

RE: Technical Support for the Study - *Microbial Activity in Silver Bow and Blacktail Creeks*

Dear Alysia,

I will consult 40 hours on the overall planning and design of the above project including the exact location of sampling sites and 40 hours on data integration for a total of 80 hours at my consulting rate of $110/ for a total in-kind match of $8,800.

Best of Luck,



Joe Griffin, P.G.

Contaminant Hydrogeologist

406 560-6060

Cox, Alysia

From: Timmer, Jackie
Sent: Monday, November 14, 2016 10:15 AM
To: Cassidy, Carleen
Cc: Gammons, Chris; Cox, Alysia
Subject: BNRC projects for Gammons and Cox

Carleen,

The Montana Bureau of Mines and Geology Analytical Lab will provide analysis for Chris Gammons and Alycia Cox BNRC projects, if funded. The analytical lab will provide a 50% discounted rate of which, can be used as cost share in each Dr. Gammons and Dr. Cox's BNRC small project proposals.

Please let me know if you need anything further.

Thanks

JACKIE TIMMER
Chief Chemist
MBMG
Montana Bureau of Mines and Geology

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