1.0 Project Proposal Summary Sheet

Project Title	Examining the pathogenicity of the microbial profile in Rapid Creek and Spring Creek

Project Period	July 1, 2015-June 30, 2017

Project Sponsor:	
Organization	South Dakota School of Mines and Technology
Primary Contact	Lisa Kunza
Title	Assistant Professor, CABS
E-mail address	lisa.kunza@sdsmt.edu
Mailing address	501 E. St. Joseph St
City, State, Zip	Rapid City, SD 57701
Telephone	605-394-2449
Fax	605-394-1232

Project Funding:	
Budget Category	Amount ($)
NPS Funds Requested	156,849
Match (cash/in-kind)	104,565
Other federal funds	0
Total Project Cost	261,414

Project Type			
Planning/Assessment: 90%	Watershed	Information and Education-10%	Groundwater

Project Location	
Watershed(s):	Rapid Creek and Spring Creek
303(d) Listed Stream	Yes No Listed Segment + AUID# SD-CH-R-RAPID_03; SD-CH-R-RAPID_04; SD-CH-R-RAPID_05; SD-CH-R-SPRING_01
HUC(s) (8 digit USGS Hydrologic Unit Codes)	01100303,01100304,01100305,01090901,01090902,01090903,01090904, 01100202,01100203,0110204
County(ies)	Pennington
Coordinates of project location	Latitude- 44.0760°N Longitude-103.2280°W

NPS Pollution Source categories to be addressed (enter percent for each applicable source)			
40	Agriculture		Silviculture
	AFOs		Hydrologic Modification
60	Urban Runoff		Construction
	Resource Extraction		Other:

NPS Functional Category (enter percent for each applicable functional category)			
5	BMP Implementation	5	Technical Assistance
10	Information and Education	10	Planning
	Watershed Assessment		Groundwater
70	Water Quality Monitoring		Other:

NPS Pollutants to be addressed (Check all that apply)			
	Excess Nitrogen		Pesticides
	Excess Phosphorus		Oil and grease
X	Sedimentation		Temperature
X	Pathogens/Bacteria		pH
	Metals		Other:
	Low dissolved oxygen		Other:

Summary Statements	
Project Goals	1) Examine the proportion of fecal coliforms and *Escherichia coli* present relative to the entire bacterial profile. 2) Estimate the proportion of the bacteria carrying pathogenic genes and pose a greater health risk. 3) Link total suspended solids to bacterial loads and pathogenicity to help inform best management practice development. 4) Public outreach of our results to connect with the landowners and local waterway users.
Project Description	To help guide future BMPs, we will establish a temporal baseline of pathogenic potential of the bacterial profiles of Rapid Creek and Spring Creek and link these estimates with total suspended solids. Although fecal coliforms and *E. coli* are commonly quantified in the monitoring of our water systems by local, state, and federal agencies, the level of pathogenicity is often overlooked. We propose to create a new monitoring metric for pathogenic potential of the contaminated water by screening the bacteria for harmful traits that can be passed even among harmless bacteria, creating the possibility for severe public health risks.

Proposal Narrative

2.0 Statement of Need

Water quality testing relies on measuring levels of bacteria that are common inhabitants of the guts of animals, either total fecal coliforms, or, more specifically, *E. coli*. However, these bacteria, although providing an indication of fecal contamination, have varying levels of harmful attributes. For example, the normal human gut strains of *E. coli* may cause little or no illness when ingested. However, the serotype *E. coli* O157:H7 has caused numerous outbreaks of severe diarrhea, sometimes resulting in hemolytic uremic syndrome, which is particularly lethal in small children. The difference between harmless, normal gut inhabitants and dangerous pathogenic organisms lies in the genes. Pathogenic *E. coli* carry genes that allow them to produce toxins, such as Shiga toxin, as well as other proteins designed to break down cells of their hosts. Identification of a water sample as carrying *E. coli* provides no information about the potential danger of consumption of the water. Everywhere in the United States, and particularly in many rural western states, there are significant problems with both human and animal contamination of recreational use waters, both in populated as well as uninhabited areas. What is unknown is the potential for outbreaks of severe illness from the resident fecal coliform population.

The more of the potentially harmful genes an organism contains, the higher the danger to human health. Unfortunately, these genes are often easily transmitted between cells, even those of very different species. Thus, it is likely that new, more pathogenic strains will be created in areas where the total bacterial count is high. The factors that affect concentration of bacteria within these waters is dependent on many factors, including the uncontrollable, such as rainfall, as well as the more manageable, such as nearby land management practices. We propose to test for the presence of these genes in contaminated waters, to determine the level of existing pathogenic potential, and to correlate the bacterial profile to total suspended solids. This analysis will help guide future BMP implementation and creation of new monitoring metrics for pathogenic potential of contaminated water, and could benefit national agencies, such as the National Park Service and the EPA, as well as South Dakota state agencies including the DENR.

2.1 Demonstrated Water Quality Need:

Rapid Creek as it traverses Rapid City, from Canyon Lake to S15/T1N/R8E, has been designated as impaired (EPA category 4A); S15, T1N, R8E to above Farmingdale (EPA category 5); above Farmingdale to Cheyenne River (EPA category 4A) in the 2014 South Dakota Integrated Report for Surface Water Quality Assessment [1]. Spring Creek, from S5, T2S, R3E to Sheridan Lake, has been designated impaired with an EPA category 5. All stretches have unacceptable levels of fecal coliform, attributable in part to urban runoff and onsite treatment systems for sewage, caused by human activity. Other sources may include cattle and wildlife. Rapid Creek in the section from above Farmingdale to the Cheyenne River has also been designated as impaired for total suspended solids. As western South Dakota becomes more popular both as a permanent residence and a tourist destination, these activities could increase and exacerbate the problem in both areas. In Rapid City, planning is underway to address the best management practices for Rapid Creek. However, Rapid Creek is not a static body. Currently, Canyon Lake drainage is underway, which may cause changes to the ecology of the creek. Upon completion of this project, the fecal coliform status of the creek could be transiently or permanently altered.

It has recently been shown that *E. coli* isolated from drinking and recreational water harbor the genes associated with the pathogenic serotype 0157:H7, the enterohemorraghic *E. coli* (EHEC) that causes severe illness including colon damage and hemolytic uremic syndrome [2]. Genes that encode the Shiga-type toxins found in O157:H7 are carried by bacterial viruses, or phages, which are carried in the chromosome of *E. coli* hosts in a quiescent, or lysogenic, state. It has been demonstrated that the presence of these lysogenic phages in the genome of the bacteria provides an advantage by preventing eukaryotic predators from eating them [3]. Environmental triggers cause these phages to begin their replication cycle and leave the host chromosome, where they can infect other *E. coli* cells. This process means that there may be an endemic source of potentially pathogenic *E. coli* in any water supply that has demonstrated levels, and absence of apparent illness is not a good indicator. In addition, bacterial populations are known to transfer DNA through other means, even between relatively unrelated species. The *stx* genes, which comprise two antigenically distinct variants, have been found not only in the coliform bacteria *E. coli*, *Enterobacter*, and *Citrobacter*, and the aquatic bacterium *Aeromonas*, but in the soil bacterium *Acinetobacter*, the food pathogens *Campylobacter* and *Shigella*, and even an insect endosymbiont, *Hamiltonella* [4, 5]). Clearly, the widespread nature of these genes indicates significant lateral gene transfer has taken place, and presumably continues to take place. In both Rapid Creek and Spring Creek, using fecal coliform counts as an indicator of water quality provides an inadequate picture of the health of the system with regards to the potential for severe human illness.

Both creeks are affected by human activity; however, the more urban nature of Rapid City and the wastewater treatment above Canyon Lake introduces a different mix of potential contaminants than the more rural, individual waste treatments (septic tanks) that affect water quality through Hill City. Both domestic and wild animals have also been shown to contain *stx*-carrying bacteria as part of their natural flora, and may also contribute to the reservoir of these genes within these systems. We propose to conduct a more extensive characterization of the bacterial population present in both systems. Monitoring total culturable bacteria, fecal coliform and specifically *E. coli* over a 18 month period will provide a baseline of microbial distribution. In addition, these numbers will be correlated with total suspended solids, which likely affect each of these numbers. Finally, the *E. coli* and fecal coliforms will be tested for the presence of the Shiga toxin genes (*stx1* and *stx2*), as well as two other virulence genes carried by *E. coli* O157:H7, hemolysin A (*hylA*) and intimin (*eae*).

[1] Pirner, S.M. (2014) The 2014 South Dakota Integrated Report for Surface Water Quality Assessment.
[2] Smith, C.J., *et al.* (2009) Correlation of shiga toxin gene frequency with commonly used microbial indicators of recreational water quality. *Appl Environ Microbiol* 75, 316-321
[3] Lainhart, W., *et al.* (2009) Shiga Toxin as a Bacterial Defense against a Eukaryotic Predator, Tetrahymena thermophila. *J. Bacteriol.* 191, 5116-5122
[4] Mauro, S.A. and Koudelka, G.B. (2011) Shiga toxin: expression, distribution, and its role in the environment. *Toxins* 3, 608-625
[5] Degnan, P.H., *et al.* (2009) Hamiltonella defensa, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. *Proceedings of the National Academy of Sciences* 106, 9063-9068

2.2 Waterbody Information: The proposed project will have local, state, and national implications. Data collection will be focused in Rapid Creek and Spring Creek both located in Pennington County, SD. Rapid Creek is impaired for fecal coliform/*E. coli* from Canyon Lake in Rapid City until the confluence with the Cheyenne River and also total suspended solids from above Farmingdale, SD to the Cheyenne River. Spring Creek is listed as impaired for fecal coliform in the section from Hill City, SD to Sheridan Lake. Detailed watershed descriptions can be found in the TMDLs for both Spring Creek (Kenner & Larson 2008) and Rapid Creek (Kenner & Smith 2010, Smith 2011)

2.3 Project maps:

Figure 1. Rapid Creek watershed (Kenner & Smith TMDL 2010). Our sampling locations are denoted using red stars.

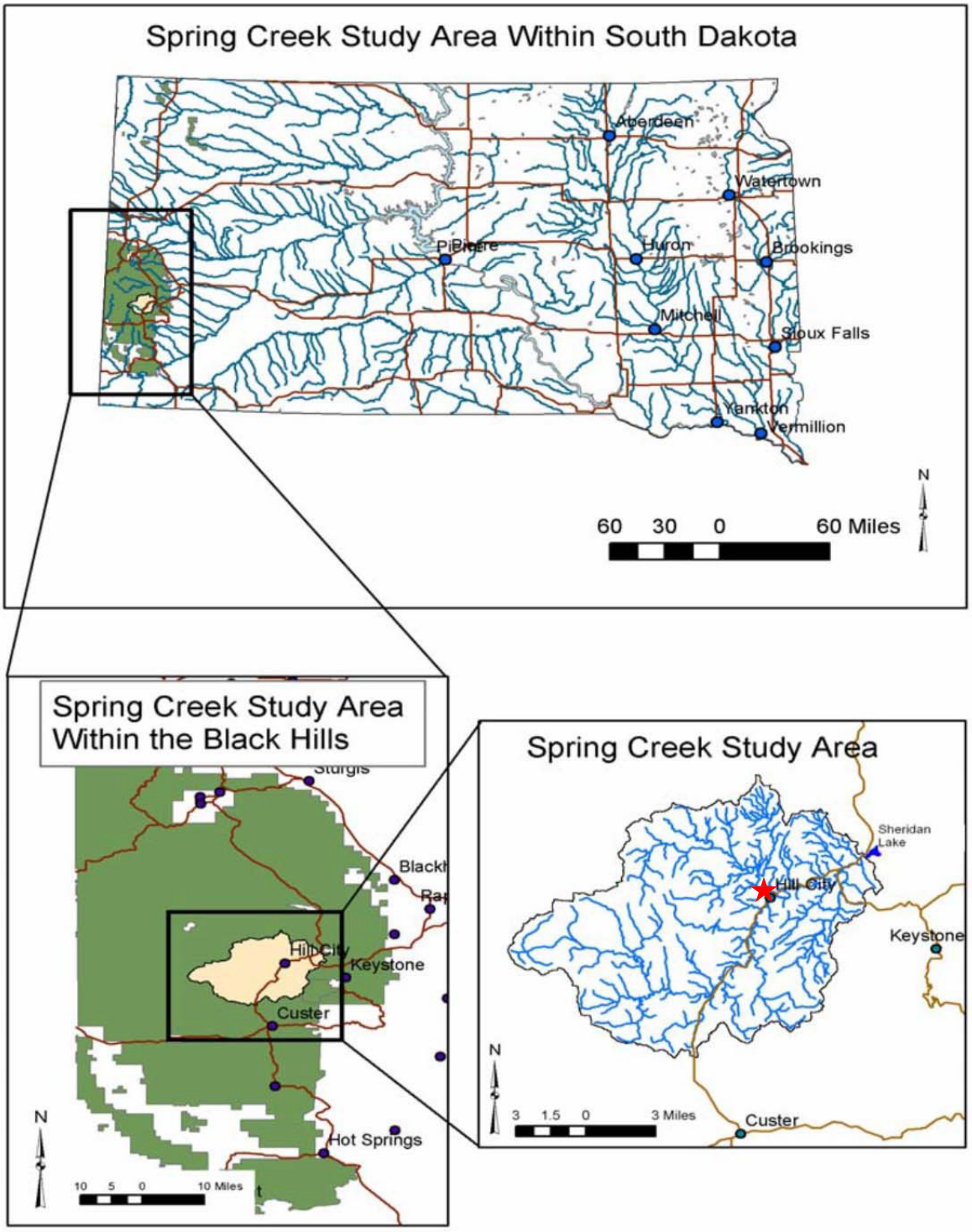

Figure 2. Spring Creek watershed (Kenner & Larson TMDL 2008) our sampling location is marked using a red star.

2.4 General Watershed Information:

Detailed watershed descriptions can be found in the TMDLs for both Spring Creek (Kenner & Larson 2008) and Rapid Creek (Kenner & Smith 2010, Smith 2011) all accessible online via the SD DENR website (https://denr.sd.gov/dfta/wp/tmdlpage.aspx).

3.0 Project Description

In this project, the pathogenicity potential of existing fecal coliforms in Rapid Creek and Spring Creek will be measured over an 18 month period and linked to total bacterial profiles and suspended solids.

3.1 Project Outcomes:

The final outcome of this project is to establish a temporal baseline of pathogenicity potential of the bacterial contamination of Rapid Creek and Spring Creek. This information will help guide future BMPs, and provide public health information regarding the safety of human exposure to the waters in these areas. Total fecal coliforms, and more recently, *E. coli* alone, have been used as an indicator of contamination. What is unknown is the relative abundance of these to the total cultivatable bacterial population. Because bacteria transmit genetic information, including pathogenicity and antibiotic resistance genes, between even unrelated species, it is important to know the total concentration, and thus potential genetic pool, in the contaminated water. It has been reported in other systems that the presence of pathogenicity genes within contaminated waters is not dependent on fecal coliform concentrations [2], as many species of bacteria are known to carry these genes. It is important to understand the distribution of these genes within the two contaminated water systems, to determine if there is a higher public health concern than is indicated by the fecal coliform alone. It is anticipated that this information will result in the creation of a new monitoring metric, and may provide a means to identify source pollutants. Our goal is not only to examine the bacterial load and pathogenicity, but help relay the information through outreach to the general public via brochures and public presentations.

3.2 Outcomes, Targets, and Tasks:

- Outcome: Comparing the potential genetic pathogenicity of contaminating bacterial as well as non-indicator bacteria with environmental factors such as seasonal changes and heavy precipitation (as indicated by total suspended solids), a baseline for public health danger will be established. The results of this project may result in creation of a new monitoring metric, and ultimately provide a molecular means for identifying sources of contamination. This, in turn, would guide creation and implementation of new management practices for reducing risks for potentially lethal outbreaks of severe human illnesses caused by pathogenic contaminating bacteria.
    - Target 1. Obtain Samples and reproducibly analyze for bacterial populations

Task # 1 : Collection of samples	
Task Description:	Water samples will be collected in triplicate from four publically accessible locations on Rapid Creek and one location on Spring Creek, monthly for 18 months.

Task Outputs:	A collection of water samples that span an 18 month period, representing seasonal fluctuations as well as precipitation changes.	
Cost:	Federal (319) Funds:	$500 Labor Costs and match are included in the overall budget
	Non-Federal Match:	$500
	Other Federal Funds:	
	Total Task Cost:	$1000

Task # 2 : Analysis of samples for total bacterial count and fecal coliform		
Task Description:	Cultivate the water samples on media for total cultivatable cfu/ml, and replicate onto differential media for fecal coliform and E. coli.	
Task Outputs:	Quarterly and final comparison of total bacterial count vs. fecal coliform and E. coli.	
Cost:	Federal (319) Funds:	$2000 Labor costs and match are included in the overall budget
	Non-Federal Match:	$1000
	Other Federal Funds:	
	Total Task Cost:	$3000

- Target 2. Identify the pathogenic potential of Rapid Creek and Spring Creek water by measuring the presence and prevalence of toxin and other virulence genes in samples collected monthly over an 18 month period. This information will be correlated with the data collected in Target 1.

Task # 3 : A PCR screen of total DNA isolated from each water sample for four pathogenicity genes		
Task Description:	Each sample will be analyzed for presence of four different genes	
Task Outputs:	Quarterly and final profiles of the presence/absence of pathogenicity genes over an 18 month period.	
Cost:	Federal (319) Funds:	$3000 Labor costs and match are included in the overall budget
	Non-Federal Match:	$2000
	Other Federal Funds:	
	Total Task Cost:	$5000

Task # 4 : RT PCR analysis of positive samples for quantification of pathogenic gene		
Task Description:	For each sample that contains one or more of the genes, the number of copies will be measured using quantitative PCR. This will then be correlated back to the bacterial load.	
Task Outputs:	Quarterly reports on the number of copies of each pathogenicity gene relative to the number of total bacteria and coliforms over an 18 month period.	
Cost:	Federal (319) Funds:	$7000 Labor costs and match are included in the overall budget
	Non-Federal Match:	$2500
	Other Federal Funds:	
	Total Task Cost:	$9500

Task # 5 : Identify specific contaminating bacteria carrying pathogenicity genes		
Task Description:	In samples with high pathogenicity potential, individual isolates will be screened for genes and identified.	
Task Outputs:	A list of bacterial species isolated carrying pathogenicity genes. This list will add to the overall profile of the contaminated water and potentially aid in source identification	
Cost:	Federal (319) Funds:	$1800
	Non-Federal Match:	$
	Other Federal Funds:	
	Total Task Cost:	$1800

- Target 3: Measure total suspended solids in water samples collected monthly over an 18 month period and correlate these to information in Targets 1 and 2.

Task # 6 : Measure Total Suspended Solids		
Task Description:	Each of the samples collected in Task #1 will be filtered, the filter dried, and weighed for TSS.	
Task Outputs:	Monthly and quarterly reports with the TSS from all samples, correlated with data from Tasks # 2-4.	
Cost:	Federal (319) Funds:	$200
	Non-Federal Match:	$
	Other Federal Funds:	
	Total Task Cost:	$200

- Target 4: Inform the public of the pathogenic potential of the bacterial populations found in Rapid Creek and Spring Creek. Landowners along these waterways should be made aware of the temporal distribution and any correlations with environmental factors, so that water exposure can be adjusted accordingly. Submit annual and final report to DENR/EPA.

Task # 7: Inform public of findings		
Task Description:	Inform scientists and landowners of the pathogenic potential found in the water through scientific and public meetings, and flyers.	
Task Outputs:	Presentations at scientific and public meetings, and creation and distribution of flyers to affected landowners	
Cost:	Federal (319) Funds:	$500 Labor costs are included in the overall budget
	Non-Federal Match:	$
	Other Federal Funds:	
	Total Task Cost:	$500

Task # 8 : Report findings to funding agency		
Task Description:	Create annual and final report of findings	
Task Outputs:	Annual and final reports to DENR/EPA	
Cost:	Federal (319) Funds:	$0 Labor costs are included in the overall budget
	Non-Federal Match:	$
	Other Federal Funds:	
	Total Task Cost:	$

3.3 Milestone Table

	Quantity	2015		2016				2017	
		July-Sept	Oct-Dec	Jan-Mar	Apr-June	Jul-Sept	Oct-Dec	Jan-Mar	Apr-Jun
OBJECTIVE 1: Sample collection and Analysis									
Task 1: Triplicate collection from 5 sites									
Product 1: Samples	270	45	45	45	45	45	45		
Task 2: Total and fecal counts									
Product 2: Report on total and fecal count at each site	90	15	15	15	15	15	15		
OBJECTIVE 2: Molecular Analyses of pathogenicity genes									
Task 3:PCR screen of 4 genes									
Product 3: Report on presence of pathogenicity genes at each site	90	10	15	15	15	15	15	5	
Task 4: RT PCR on selected samples									
Product 4: Report on correlation of # copies of genes to total bacterial count	up to 90			up to 15	up to 15	up to 15	up to 15	up to 15	up to 15
Task 5: identification of bacterial carrying pathogenicity genes									
Product 5: List of positive bacterial species	90			15	15	15	15	15	15
OBJECTIVE 3: Total suspended solids									
Task 6: Analyze TSS in each sample									
Product 6: Report on TSS	270	45	45	45	45	45	45		
OBJECTIVE 4: Outreach									
Task 7: Inform public									
Product 7: Public and scientific meetings	6		1		1		1		3
Product 8: Creation and distribution of flyers	1								1
Task 8: Reporting									
Product 9: Annual and Final report to DENR/EPA	2				1				1

3.4 Project Management and Tracking

The project will be led by two SDSMT professors, Dr. Lisa Kunza and Dr. Linda DeVeaux. Dr. Kunza is an assistant professor in the Department of Chemistry and Applied Biological Sciences and Program of Atmospheric and Environmental Sciences. Dr. Kunza has 11 years of experience studying water quality and aquatic ecology. Dr. DeVeaux, associate professor in the Department of Chemistry and Applied Biological Sciences and Associate Director of the Biomedical Engineering Program, has 35 years of experience in microbiology and molecular biology. Combined the professors have mentored 28 graduate students and 75 undergraduate students in research activities, and together will direct the graduate students working on this project. The graduate student who will be carrying out the molecular and microbiological analyses, Kelsey Murray, is in the Biomedical Engineering Ph.D. program at SDSMT with Dr. DeVeaux as her major advisor. She has extensive experience in molecular biology techniques, and is a South Dakota resident. Another graduate student involved in sample collection and suspended solids analysis will have Dr. Kunza as a major advisor.

Monthly samples will be filtered onsite for total suspended solids following standard procedures in the laboratory. Sterile samples will be collected simultaneously in triplicate, and stored on ice or refrigerated for no more than 24 hours prior to analysis. For total bacteria, samples will be diluted and plated, or filtered first, depending on concentration. Parallel samples will be filtered for DNA isolation prior to PCR amplification. PCR will be carried out according to published protocols. Significant findings will be presented at scientific meetings and submitted for peer-reviewed publication.

3.5 Permits

No permits for water sampling are required, as all sites have public access.

4.0 Coordination Plan

4.1 Cooperating Organizations

All faculty and students are from South Dakota School of Mines.

4.2 Local Support for the Project

Both West Dakota Water Development District and the National Park Service-Northern Great Plains Inventory and Monitoring Network have expressed interest in the results from our work. Please see attached letters of support in appendix.

4.3 Duplicate Effort

Although quality is monitored by local and state agencies and federal agencies through testing for fecal coliform/*E. coli* and TSS, there is no analysis of pathogenicity potential being carried out The sampling efforts do not currently overlap.

4.4 Assumption of the Responsibilities of Other Entities

This analysis effort is not the responsibility of any other entity.

5.0 Evaluation and Monitoring Plan

5.1 Monitoring Strategy

The data collected are part of the monitoring. See Section 3.2.

5.2 Sampling and Analysis Plan

The sampling and analysis will mimic those in place by state agencies for water quality assessment by fecal coliform/*E. coli* measurement. Additional testing will use standardly accepted microbial and molecular techniques. Linking fecal coliform/*E.coli* samples with sediment load via TSS estimates at the same locations and time of sampling will be necessary. Further information will be provided upon request.

5.3 Quality Assurance Project Plan

A quality assurance program plan will be developed prior to project implementation if project is selected for funding.

5.4 Data Collection, Management and Analysis

The graduate students will collect the data, and together with the professors, will analyze, manage and report. All parties will participate in preparing manuscripts for peer-reviewed publication. Additionally, all data will be archived on the SDSM&T server, which is backed up daily. For more specific information about data collection see section 3.2.

5.5 Models

No models are necessary for the interpretation of data. Standard statistical methods will be used to analyze the data.

5.6 Operation and Maintenance

Our findings will inform future BMPs.

6.0 Information and Education

6.1 Information and Education Activities

Tasks are outlined in 3.2. Briefly, we plan to give multiple public presentations and distribute flyers to inform the public about our findings. Furthermore, our graduate students will use this research in partial fulfillment of their degree programs. Results will also be disseminated via presentations at scientific meetings and publications in peer-reviewed journals.

7.0 Budget and Budget Justification

Project Name: Examining the pathogenicity of the microbial profile in Rapid Creek and Spring Creek					
Budget: Segment: Date: June 1, 2015 Through May 31, 2017					
ITEM	Year 1	Year 2	Total	319-EPA	SDSMT
	2015-2016	2016-2017			
Personnel Support					
Senior Personnel					
PI- Dr. Lisa Kunza (8% effort academic year, 0.5 month summer salary + fringe)	$12,408	$12,717	$25,125	$10,143	$14,982
Co-PI- Dr. Linda DeVeaux (8% effort academic year, 0.5 month summer salary + fringe)	14,446	14,808	29,254	11,810	17,444
Other Personnel					
Graduate Student Support	46,796	47,965	94,761	72,689	22,072
Tuition Remission	12,308	12,614	24,922	20,317	4,605
Travel:					
Vehicle: 75 mi/mo. @ $0.37 per mi (12 mos. in Yr 1; 6 mos. in Yr 2)	333	167	500	500	0
Subtotal Personnel & Travel Support	86,291	88,271	174,562	115,459	59,103
Supplies:					
Research Supplies	10,000	10,000	20,000	15,000	5,000
Informational Outreach supplies	0	500	500	500	0
Total Direct Costs:	96,291	98,771	195,062	130,959	64,103
SDSMT Indirect Costs (39% Modified Total Direct Costs)*	32,753	33,599	66,352	25,890	40,462
Total Project Cost:	$129,044	$132,370	$261,414	$156,849	$104,565
Match:					
Match: Project Totals For Match					$104,565
Match Percentages:					40.00%

Examining the pathogenicity of the microbial profile in Rapid Creek and Spring Creek

Budget Justification

A. & B. PERSONNEL: The PI, Dr. Lisa Kunza, and the co-PI, Dr. Linda DeVeaux, each request 0.5 summer month's salary in each year for their effort in the project. They will each contribute approximately 13% of their academic time each year as matching support ($26,233 – Year 1 and $26,889 – Year 2). One graduate research assistant will work on the project part-time (20 hours per week) during the academic months and full-time (40 hours per week) during the summer months. His/her Summer 2015 and Fall 2016 salary, fringe benefits and tuition will be provided by the BME program as matching support ($13,300 – Year 1 and $13,377 – Year 2). An additional graduate student will work on the project 10 hours per week during the academic months and 20 hours per week during the summer months. The salaries that will be charged for this work will be those regularly approved for the individuals involved by the governing body of the South Dakota School of Mines and Technology; namely the Regents of Education of the State of South Dakota. The rates for FY 2015 (July 1, 2014 through June 30, 2015) are based on current contractual agreements for FY 2015 and assume an increase of 2.5% per fiscal year thereafter. Rates for students are approved for the period of mid May 2014, through mid May 2015, and assume an increase of 2.5% per year thereafter. If increases exceed that amount, effort stated will need to correspond with salary.

Eligible personnel earn vacation at the rate of 10 hours per month if they have 15 years or less of service and 13.33 hours per month if their period of service exceeds 15 years. This vacation is handled in the budget by including the appropriate accrual adjustment in the salary rate. This procedure is utilized for all sponsored research to ensure that each project pays only its pro rata share of the vacation authorized.

FRINGE BENEFITS: Fringe benefits have been budgeted at 23.77% of salaries and wages for faculty and staff personnel and 7.79% for students. The benefits consist of contributions to social security, the unemployment insurance program, the workmen's compensation program, the flexible benefit fee program, a group insurance program and matching contributions to the state employee retirement program. Only the actual costs of the fringe benefit programs are charged to the project.

C. EQUIPMENT: No major equipment (with dollar amount exceeding $5,000) is requested. All necessary equipment is available at South Dakota School of Mines and Technology.

D. TRAVEL: Domestic travel funds will be used by the PI, co-PI and/or the graduate students to collect samples and travel to field sites during the project. An estimated 75 miles will be travel during each of the first 18 months of the project. Travel costs are based on current estimates and will be conducted in accordance with the State of South Dakota Travel Regulations.

E. PARTICIPANT SUPPORT COSTS: No funds are requested.

G. OTHER DIRECT COSTS:

1. Materials and Supplies: Funds are allocated for research materials and supplies, mainly molecular biology and microbiology chemicals and other consumables, based on historical usage in the PI's and co-PI's labs for similar types of work, and informational outreach supplies. $2,500 will be provided by the PI and co-PI's faculty start-up funds for supplies as matching support. Expenditures reflected in these categories are based on best estimates now available.

6. Other: Funds are allocated for tuition remission of sponsored graduate students, based on the current rate of $400.15 per credit hour, with an increase of 2.5% per year thereafter. Expenditures reflected in these categories are based on best estimates now available. In no case will the project be charged for more than actual costs incurred.

I. INDIRECT COSTS (F&A): The latest indirect cost rate approved by the cognizant government audit agency for the South Dakota School of Mines and Technology is 39.0% of modified total direct costs. This rate has been used in the budget calculations for both 319-EPA funds and matching support. The cognizant government audit agency for the institution is:

Director, Division of Cost Allocation
DCA Western Field Office
Department of Health and Human Services
90 7th Street, Suite 4-600
San Francisco, CA 94103-6705
HHS Representative: Jeanette Lu
Telephone Number (415) 437-7820

Appendix- letters of support

WEST DAKOTA
WMl:R
1Jevefopment 1JiJ'frict

September 30,2014

Bany A. McLaury
SDDENR
Joe Foss Building
523 East Capitol
Piene, SD 57501-3182

RE: Bacterial loading and suspended solids in Rapid Creek

Dear Mr McLaury,

I am writing in support of Dr. Lisa Kunza's proposal to study bacterial loading and suspended solids in Rapid Creek.

Recreational water bodies face potential human and animal bacterial contamination. Identifying potential for outbreaks of severe illness from the resident fecal coliform population is a crucial first step in prevention.

From my understanding, this research will ultimately help recognize best management practices that will alleviate manageable bacteria loading and examine better mechanisms for monitoring.

Finally, the public relations component of this proposed study wan-ants its own letter of support. It is vital that we are made aware of our potential impacts as well as empower our residents to recognize their ability to help protect our valuable water resources.

As always, please contact me should you have any questions regarding this letter of support.
605.415.3928

Thank you,

J) , W

Denise Livingston
Manager
dlivingston@rcac.org

Cc: Dr. Lisa Kunza
File

National Park Service
U.S. Department of the Interior

Northern Great Plains
Inventory and Monitoring Network

231 East Saint Joseph St.
Rapid City, SD 57701

605-341-2807 phone
605-341-7192 fax
nps.gov/im/units/ngpn

Date: September 30, 2014 **Prepared** by: Marcia Wilson, Network Ecologist

Topic: Letter of Support for Proposal Submitted by Dr. Lisa Kunza and Dr. Linda DeVeaux

Mr. Barry A. McLaury
South Dakota Department of Environment and Natural Resources
Joe Foss Building
523 East Capitol
Pierre, SD 57501-3182

Dear Mr. McLaury,

This is a letter of support for a proposal submitted by Dr. Lisa Kunza and Dr. Linda DeVeaux from the South Dakota School of Mines and Technology. The proposed work will provide bacterial community baseline data for Rapid Creek in and around Rapid City. In addition, the work will examine the pathogenicity of these bacteria and the linkage of Total Suspended Solids to the bacterial loads.

We have asked Lisa to assist us with *E. coli* and fecal coliform work that was done at Wind Cave National Park. Not only will her proposed work assist the State but it will also help us with *E. coli* and fecal coliform issues in our Black Hills parks. Therefore, I strongly support this project.

I can be reached at 605-341-2803 if you have any questions.

Sincerely,

Marcia

Marcia H. Wilson, Ph. D.