worldwaterforum
Metropolitan Water District of Southern California College Grant Program

LOMA LINDA UNIVERSITY

COLLEGE:
Loma Linda University School of Public Health

PROJECT TITLE: A comparative microbial composition assessment of recycled water originating from stormwater and wastewater.

FACULTY PROJECT MANAGER and GRADUATE STUDENT PROJECT MANAGERS:
Ryan G. Sinclair PhD, MPH
Rose Mutiso
Thomas Hile
Ed Tsui

PROJECT STRAND:
Local

2. ONE-PAGE SUMMARY

Study Title: *A comparative microbial composition assessment of recycled water originating from stormwater and wastewater.*

Project description

This study will include the comparison of the effectiveness of the Quantitray method to culture-based methods (mTEC and mEI) for the enumeration of *E.coli* and enterococci as indicator bacteria for fecal pollution in stormwater and wastewater. It will assess the microbiological characteristics of raw and treated urban runoff water coming into and out of SMURRF alternative water system (AWS) site over several months. SMURFF samples will include: Raw stormwater, filtered/UV treated water and the finished chlorinated water. For comparison, wastewater from the Eastern Municipal Water District (EMWD) will also be sampled. Three samples will be collected, one from each of the following treatment stages: Raw Sewage, Secondary treated wastewater, and Tertiary treated wastewater for reuse. Samples from the two sites will be analyzed in the Environmental Microbiology Research Laboratory (EMRL) in Loma Linda University. Analysis will compare the finished storm water with the finished tertiary treated wastewater. The resulting data will be used to test the above listed hypotheses. An HPLC will be used for the finished waters to determine concentrations of the USEPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs). The findings can form the basis for future regulatory activity regarding AWS, as there are currently limited standards for such water systems.

3.CONTACT INFORMATION

College	Loma Linda University
Department	Research Affairs
Make Check Payable To:	Loma Linda University

3.A.

Application Strand	Select One
LOCAL Project Name	A comparative microbial composition assessment of recycled water originating from stormwater and wastewater.

3.B.

Faculty Project Manager	Ryan G. Sinclair PhD, MPH	
Title	Assistant Professor.	
Department	Environmental Microbiology	
Campus Address	24951 N. Circle Drive, Suite 2104, Nichol Hall	
Telephone / Email Address	909-558-400 Ext.85633	rsinclair@llu.edu

3.C.

Student Project Manager	Rose Mutiso, Thomas Hile, and Tsui, Cheng-Hsiao.	
Undergraduate or Graduate	Graduate	
Department	School of Public Health and School of Medicine	
Cell Phone / Email Address	Rose Mutiso-909-991-5418, Thomas Hile-909-646-0433, Tsui Cheng Hisao- 310-720-7843	Rose Mutiso- rmutiso@llu.edu, Thomas Hile- thile@llu.edu, Tsui Cheng Hisao chtsui@llu.edu

3.C.

Contracts Manager / Officer	Aleta Savage	
Title	Director of Financial Management	
Department	Research Affairs	
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3.E. PROJECT MANAGEMENT TEAM

	NAME	TITLE / ORGANIZATION	ADDRESS	PHONE & EMAIL
1	Dr. Ryan Sinclair	Project faculty leader and consultant in water quality, MWD experience/ LLU SPH	24951 N. Circle Drive, Loma Linda, CA, 92354	909-558-4000 x85633 rsinclair@llu.edu
2	Rose Mutiso	Student Co-Lead / LLU SM	11065 campus Street, Loma Linda, CA, 92354	909-991-5418 rmutiso@llu.edu
3	Thomas Hile	Student Co-Lead / LLU SM	11065 campus Street, Loma Linda, CA, 92354	909-646-0433 thile@llu.edu
4	Tsui Cheng Hisao	Student Co-Lead / LLU SPH	24951 N. Circle Drive, Loma Linda, CA, 92354	310-720-7843 chtsui@llu.edu

3.E. MEMBER AGENCY(IES) / LOCAL WATER AGENCY(IES)

	NAME	TITLE / ORGANIZATION	ADDRESS	PHONE & EMAIL
1	Jayne Joy, P.E.	Director of Environmental & Regulatory Compliance/ Eastern Municipal Water District	2270 Trumble Rd., Perris CA 92570	951-928-3777 x6241 joyj@emwd.org
2	Tom Watson	Water Resources Protection Program	1212 5th St., Santa Monica, CA 90401	310-458-8235 tom.watson@smgov.net

(Jayne Joy's Email attached at the end of application)

4. ORGANIZATIONAL BACKGROUND

Loma Linda University (LLU)

LLU is a nationally-known health sciences institution with over 3,000 students, situated in one of the fastest growing areas in the US. The University is affiliated with the Loma Linda University Medical Center (LLUMC). LLUMC operates some of the largest clinical programs in the U.S. in areas such as neonatal care, and is recognized as the international leader in infant heart transplantation and proton treatments for cancer. LLU has Schools of Medicine, Dentistry, Public Health, Nursing, Pharmacy, Allied Health Professions, Religion and a School of Science and Technology (SST). While LLU is a small private university, it has a long history of excellence in teaching health science professionals. (it recently celebrated its centennial), and a demonstrated commitment to research addressing the needs of high-risk populations

LLU has received funding through federal (CDC, NIH), state, community and private funding sources to study a variety of issues such as teenage pregnancy, air quality, and health disparities. External support for these research activities is currently amounting to $35.7 million, with $29.4 million in total competitive extramural funding and $15 million in NIH funding.

School of Public Health

The LLU School of Public Health has been in existence since 1967, and currently offers master's level degrees in biostatistics, epidemiology, environmental health, global health, health policy and leadership, nutrition, preventive care, public health practice, and business administration. Doctoral degrees are given in epidemiology, global health, health policy and management, health education, nutrition, and preventive care. Research focuses on diabetes management and obesity prevention for Latinos, and prostate cancer education for African American men. At the request of concerned San Bernardino residents, the school submitted and won a 2010 grant from the Southern California Air Quality Management District to study the health effects of rail yards on residents living nearby.

Environmental Microbiology Research lab (EMRL)

The LLU EMRL is housed under the school of Public health. It will provide the space for the research analysis.The lab has been used for water-related researched before and therefore it already has most of the facilities that are required for the proposed project such as: the Quanti-tray sealer, an autoclave, lab-glassware, fridges, incubators and an ample bench working space.

Department of Earth and Biological Sciences (EBS).

The Department of Earth and Biological Sciences in the School of medicine offers Undergraduate degrees in Environmental Sciences and in Geology. Graduate degrees include a MS and a PhD in Biology, a MS in Geology, and a PhD in Earth Sciences.

The research interests of the Biology program faculty and students include: conservation biology, ecological physiology and venom expenditure, marine invertebrates, Behavioral Ecology, Herpetology, and sea turtle biology. Faculty and student research in the Geology program focuses on: sedimentology, paleontology, and paleoenvironmental reconstruction. Students and faculty of the program consistently publish their research findings in international, peer-review research journals, and present their research at scientific meetings around the world.

5. CERTIFICATE OF ATTENDANCE

6. PROJECT DESCRIPTION

1. Water Related Issue:

Our project will address the current drought by optimizing the testing methodology for alternative water systems (AWS). We will assess the chemical and microbiological make-up of urban runoff coming into and out of the Santa Monica Urban Runoff Recycling Facility (SMURRF). We will compare this to similar recycled water coming into the Eastern Municipal Water District's Moreno Valley wastewater treatment system. Use of recycled water will help to offset the use of drinking water for non-potable uses like flushing toilets, landscaping, and washing cars. This project is to ease the transition towards AWS by recommending feasible microbial testing routines that can be used by small city governments with cost-efficient decentralized systems.

There are several microbial monitoring methods that are used to evaluate the quality of the treated stormwater and wastewater. Many studies have evaluated the IDEXX Quanti-tray method (IDEXX, Ontario, CA)(Bain et al. 2015) for its ability to accurately culture *E.coli* and *Enterococcus* bacteria for recreational waters, marine waters, tropical and fresh waters. There is only limited data (Bitton 2005) on the use of the Quanti-tray method for enumerating indicator bacteria in urban storm runoff and recycled wastewater. Our project seeks to fill that gap by evaluating the effectiveness of this method in enumerating indicator bacteria in both raw and treated storm runoff as compared to the effectiveness of the culture-based filtration methods of Membrane Thermotolerant *E. coli* (mTEC) and Membrane *Enterococcus* Indoxyl-B-D Glucoside (mEI) (Eaton and Franson 2005).

We will test the IDEXX Quanti-tray method because it could represent a more cost and time efficient solution for water recycling facilities. The Quanti-tray method is relatively

simple, not labor intensive and costs less when compared to the filtration methods, mTEC and mEI that are commonly in use (Budnick, Howard, and Mayo 1996).

2. *Local Problem*

Southern California is in the fifth year of a drought (Anonymous 2015).We chose a local project relevant to the drought in Southern California. We address the drought through our project that validates a test methodology to better evaluate water treated from AWS for non-potable uses. The SMURRF is an excellent pilot system in an urban area to integrate an AWS that also protects the coastal waters from direct storm runoff pollution (Antich, Salgaonkar, and Gobas 2000). Additional AWSs similar to the SMURRF can be used to offset potable drinking water demand while decreasing nutrient pollution to the ocean.

The use of decentralized wastewater management systems offers significant advantages including being close both to the source of wastewater generation and to potential water reuse applications.This reduces the energy cost because water is pumped over short distances as opposed to centralized systems (Gikas, and Tchobanoglous 2009). Developing countries lack both the funding to construct centralized facilities and the technical expertise to manage and operate them. Alternatively, the decentralized approach for wastewater treatment allows for flexibility in management.The decentralized system is not only a long-term solution for small communities but is more reliable and cost effective (Massoud,Tarhini, and, Nasr.2009) The use of alternative water sources in California is still minimal for several reasons. One reason is the concern for public health risks associated with the use of such water for both non-potable uses and secondly because there is limited regulation and guidelines to govern standardization of alternative water sources within the state. Lack of clear information on the effectiveness of the technologies used to recycle and treat stormwater contributes to the fear of using alternative water sources.

To ensure that similar decentralized AWS projects like SMURRF are built, we propose validation of a simple, rapid, (Anonymous 2015) and economical method of detecting the indicator bacteria in wastewater and stormwater. There is a need to evaluate the IDEXX Quanti-tray method to determine if it is the most appropriate technique to use locally. If the technology is validated for California AWS, it can be (Anonymous 2015) replicated in other parts of the country and globally.

3. *Technology as a Content Strand*

Our research will focus on Technology as the content strand. The main objective is to assess the effectiveness of the Quanti-tray method in enumerating *E.coli* and enterococci as indicator bacteria for fecal pollution in urban runoff stormwater and in wastewater. We will also compare

the effectiveness of the Quanti-tray method to the culture-based methods: mTEC (Membrane Thermotolerant *E.coli*) and mEI (Membrane Entoerococcus Indoxyl-B-D Glucoside).

4. Research and Data Collection:

Samples of stormwater and wastewater will be collected from two water recycling facilities in Southern California: The Santa Monica Urban Runoff Recycling Facility (SMURRF), and the Eastern Municipal Water District (EMWD) respectively. The samples will then be shipped on ice to LLU EMRL for analysis within 6 hours.

Data collection will involve four trips to each of the sampling sites. One sample from the SMURFF will also be collected from the raw influent site during a rain event. The other four sample days are during dry weather. Water samples from the EMWD will be collected from the tertiary stage of treatment. A High performance liquid chromatography (HPLC) analysis will be used for the finished waters to determine concentrations of the USEPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) (Anonymous 2015)

Samples will be prepared and analyzed using three different procedures as outlined below (SMURRF QAPP)

1. Total coliform, E. coli, and enterococci enumeration- IDEXX Most Probable Number Method (Quanti-tray)
2. Method 1603, mTEC (Membrane Thermotolerant *E.coli*)
3. Method 1600, mEI (Membrane Entoerococcus Indoxyl-B-D Glucoside)

5.Anticipated outcome

Short term

- Data to determine whether the Quanti-tray method is more effective in enumerating the indicator microorganisms.
- Microbial validation and risk reduction for recycled water use in SMURRF / EMWD.

Long term

- To have data available to advocate for more AWS to be approved in CA or water quality rule standardization (of microbial methods).
- Offset use of drinking water for non-potable uses
- Reduce ocean and surface water pollution from direct runoff.

6.Projection benefits

We project that adoption of the Quanti-tray method for the enumeration of indicator microorganisms in both wastewater and stormwater runoff will reduce water treatment costs by $ 4,320 per year. The processing of 12 samples of mTEC and mEI per year costs approximately $6480 while the processing of 12 samples of comparable Quanti-trays costs $2,160 per year saving $4,320 per year.

The main benefit of this project is that it will reduce the cost of water treatment for non-potable uses in California. The proposed Quanti-tray method is economically cheaper than the culture-based methods currently in use, and therefore if it is adopted, the recycling facilities could recycle more water than their current volumes, with low operational costs. This will

encourage more recycling facilities to be set-up within the state and the end result is that, there will be additional alternative sources of water than we currently have, and this will make it easy for the public to access such water for non-potable uses. The IDEXX Quanti-tray method cost is approximately $10 per sample assay while the comparable mTEC costs $30 per sample. (Fisher Scientific, Ontario, CA).

The Quanti-tray method will provide wastewater treatment facilities with a rapid and faster alternative for measuring *E.coli* and *Enterococcus* in wastewater compared to the filtration and multiple-tube methods, because it has a short incubation period of 24 hours compared to 48 hours required by the filtration methods and requires less time per sample for setup, reading, and recording of results (Budnick, Howard and Mayo.1996). This factor will enable health officials to test any recycled water for contamination in the shortest time and take the appropriate measures towards ensuring water safety (Budnick, Howard, and Mayo.1996)

Quanti-tray is organism-specific method which suppresses non-coliforms from growing and only favors the target organisms. It detects a single, viable organism per sample and it can detect down to one organism per 100 ml. Due to this increased specificity and sensitivity (Budnick, Howard, Mayo. 1996). The Quanti-tray method will be more accurate in enumerating fecal coliforms and avoiding the false positives that can be associated with nutrient-rich methods that encourage growth of both target and non-target organisms.

Currently there is limited published literature and data on the effectiveness of the Quanti-tray method in enumerating *E.coli* and *Enterococcus* in stormwater and wastewater. It is for this reason that we content that our once our project is adopted, our study data will form an integral part of making guidelines for the general standards that should be adopted by the wastewater and runoff recycling facilities.

The data obtained from this study can assist in identifying the best management practices for AWS that result in minimum microbial growth and pathogen persistence. Having compared the effectiveness of the different methods of enumerating indicator microorganisms, this study will also enable the stakeholders to make informed decisions on the most economical and technologically-effective methods of stormwater and wastewater treatment.

Team experience and technical capabilities

Several team members will be involved in accomplishing this project by taking specific roles as outlined below.

- Experience:

  - Dr. Ryan Sinclair has worked on various public health-related projects and received funding from the Metropolitan Water District's' World Water Forum previously. He is an environmental microbiologist with a focus on human health exposure assessment.
  - Rose Mutiso has worked as research assistant in the EMRL in LLU for a water- related study.
  - Thomas Hile has a good experience in microbiology.

- Tsui Cheng Hisao has a strong background in public health.

- Roles and responsibilities:
  - Dr. Ryan Sinclair: EMRL Lab project advisor, networking, project liaison, project partner relations (EMWD, MWD, LACDPH, SMURRF, USEPA).
  - Rose Mutiso: Planning, coordinating data management and analysis, reporting the study findings.
  - Thomas Hile: Assist with Sample processing in the LLU ERML lab
  - Tsui Cheng Hisao: Assist with Sample processing in the LLU ERML lab

Project Schedule with key milestone dates and deliverables with measurable outcomes.

Milestone	**Deliverable**	Dates	**Measurable Outcome**
Water sampling at the SMURFF and processing in lab	Have data recorded in lab notebook	1/2016, 2/2016, 3/2016, 4/2016	Observed successful lab process and completion of sampling
Water sampling at the EMWD recycling facility and processing in lab	Have data recorded in lab notebook	5/2016, 6/2016, 7/2016, 8/2016	Data for samples from both recycling facilities.
Data cleaning	Have data compiled to electronic database	9/2016,10/2016	Data is available to begin analysis
Data analysis	Run ANOVA, t-tests and other statistical tests	11/2016, 12/2016,	Statistical interpretation of the results.

Final Report	Report containing results of the comparison between Quanti-tray and the mTEC and mEI.	1/2017,2/2017,3/2017,4/2017	Data stored in both the lab book and electronic storage device
Poster presentation at a scientific conference	Present the study findings at a scientific conference	5/2017	Public's change of attitude towards the use of recycled water and the dangers of surface water pollution
Publication	Identify an appropriate journal and submit the research.	6/10/2017	A peer-reviewed and published paper.

7. FINANCIAL CRITERIA

The LLU will provide Matching Funds Requirement 60% of total grant award which includes: **In-Kind contributions:** Total percentage of time devoted to the project by faculty = 5% Equipment – Microbiology lab use, HPLC, microbial assay, physical parameters analysis = $3,800 with a total= $6,000. The administrative (College) Overhead is $909: a 10% overhead fee applied to the modified Total Direct Costs (MTDC)

BUDGET OVERVIEW

DESCRIPTION	AMOUNT	NOTES
GRANT FUNDS REQUESTED	9,995	N/A
ADDITIONAL SOURCE OF FUNDS	6000	In-Kind contributions
PROJECT TOTAL	15,995	

BUDGET BREAKDOWN:

Direct Costs	COMPUTATION					
BUDGET ITEM DESCRIPTION	**Price/ Rate**	**Unit**	**Qty**	**MWD/ WWF**	**LLU Match**	**Total Costs**
SALARIES AND WAGES						
To support student time in lab, field and for reporting.	$14.0	per hour	276	$3,862	-	$3,862
SUPPLIES / MATERIALS						
$1,500 to purchase colilert media, enterolert, mEI, mTEC and other microbial media for indicator organisms. $800 for consumables to run the HPLC and $1,500 for COD/TOC and other physical parameters. $1,000 is matched by LLU for supply of incubators, sample collection bottles, coolers, glassware and other consumables.				$3,800	1,000	$4,800
TRAVEL						
$625 for personal vehicle travel for 12 trips to treatment plants and the MWD headquarters. Mileage reimbursement is the LLU rate of 45 cents per mile.	$0.45	miles	1388	$625	-	$625
PUBLICATIONS						
$300 for peer-review publication.				$300	-	$300
CONFERENCE PRESENTATION						
$500 for conference registration of students to present results at the 2017 IWA Health-Related Water Microbiology conference.				$500	-	$500
EQUIPMENT						
LLU will match the $5,000 towards HPLC equipment rental.					$5,000	$5,000
Subtotal				$ 9,087		$9,087
INDIRECT COSTS						
A 10% overhead fee applied to Modified Total Direct Costs.				$909	-	$909
TOTAL				**$ 9,995**	**$ 6,000**	**$15,995**

References.

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Bain, Robert E. S., Claire Woodall, John Elliott, Benjamin F. Arnold, Rosalind Tung, Robert Morley, Martella du Preez, et al. 2015. "Evaluation of an Inexpensive Growth Medium for Direct Detection of Escherichia Coli in Temperate and Sub-Tropical Waters." *PloS One* 10 (10): e0140997. doi:10.1371/journal.pone.0140997.

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Eaton, AD, and MAH Franson. 2005. *Standard Methods for the Examination of Water & Wastewater*. American Public Health Association; American Water Works Association. http://books.google.com/books?id=buTn1rmfSI4C

8. SIGNATURE BLOCK

	NAME / TITLE	SIGNATURE	DATE
FACULTY PROJECT MANAGER	Ryan Sinclair		12/21/2015
MEMBER AGENCY/LOCAL WATER AGENCY	Jayne Joy	See attached email	12/21/2015
STUDENT PROJECT MANAGER	Rose Mutiso		12/21/2015

Note of collaboration.

-----Original Message-----
From: Joy, Jayne [mailto:joyj@emwd.org]
Sent: Wednesday, December 16, 2015 9:18 AM
To: Sinclair, Ryan (LLU)
Cc: Mutiso, Rose (LLU)
Subject: RE: Contact Info

Ryan,
Thank you for the writeup, EMWD will be happy to support your proposal.
I can help you coordinate with EMWD for this effort. Let me know when you are ready to get started!
Merry Christmas!
Jayne