My Summer in India

Exploring Water Treatments Practices and Culture

Report Submitted to:

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By: LILLIANNE A. BROWN
Iowa City, IA, USA

Internship completed at:
M.S. Swaminathan Research Foundation (MSSRF)
Chennai, India

The World Food Prize Foundation
1700 Ruan Center
666 Grand Avenue
Des Moines, IA 50309 USA
wfp@worldfoodprize.org

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Acknowledgements

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An enormous thank you to Professor M.S. Swaminathan and Mina Swaminathan for opening MSSRF and their home to me. Their words and wisdom will never be forgotten. Thank you to Dr. Parida for leading all of the staff and scholars at MSSRF and giving me my project assignment. Thank you to Dr. Rajalakshmi for coordinating my stay at MSSRF and helping me when needed. Thank you to Dr. V.R. Prabavathy for all of her help and guidance within my project, as well as opening her lab and home to me. My upmost thanks go to Purushothaman and all of the PhD scholars at MSSRF for helping me with my research. Without my *Akka* (sisters) and *Anna* (brothers) at MSSRF my internship wouldn't have been successful.

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Finally, I would like to thank my family, my friends, and all of my co-workers at New Pioneer Food Cooperative. Your support before, during, and after my internship was crucial to its success.

I. Personal Introduction

Growing up in Iowa City, IA, I was largely isolated from agriculture practices even though I live in the heart of America's bread basket. I grew up not knowing the practices or livelihood of Iowa farmers, let alone the challenges that small holder farmers face abroad. My freshman year of high school, I started working on a composting project which opened my eyes to environmental problems that Iowa and the U.S. face. Then in my sophomore year I participated in the Iowa Youth Summit. This opened my eyes to some of the challenges that farmers overseas faced, as I studied how fertilization and education can affect the Ethiopian small holder farmer; as well as learning from the topics that my peers discussed that day. After learning about some of the challenges that farmers face abroad, I looked for ways to help within my community. My sophomore and junior year I participated in and lead a local program that helps address food and economic insecurity within the Iowa City area.

After attending the Iowa Youth Institute, I was invited to attend the Global Youth Institute in the fall of 2013. I participated in the three day symposium and felt empowered leaving. The event left me not only more knowledgeable about the challenges faced by farmers around the world, but also made me realize that I can't just stand still. I have to help. I left feeling sad about where we currently are but empowered and hopeful to make a change. This led me directly to apply for the 2014 Borlaug-Ruan Internship. The internship seemed like the next logical step for me. It gave me the opportunity to lend a helping hand, experience the problems talked about firsthand, and continue in my path to helping end world hunger. I was accepted as a 2014 Borlaug-Ruan intern and placed to study at the M.S. Swaminathan Research Foundation (MSSRF) in Chennai, India. I left for India in June of 2014 with one thing certain: my life and perspectives of world hunger would never be the same.

II. Research Introduction

2.1 M.S. Swaminathan Research Foundation

Professor M.S. Swaminathan was the first recipient of the World Food Prize, winning it in 1987. Following this great accomplishment, he used the prize winnings to establish and create the M.S. Swaminathan Research Foundation (MSSRF) in 1988. MSSRF is a NGO, nestled in the institutional district of Chennai, India.

Since MSSRF was founded, it has worked to help solve food security issues within India through its bottom up, pro-poor, women empowering programs. The foundation aims to help solve these problems through the development and use of new agriculture technology and educate farmers to correctly and effectively use it. The foundation works towards helping all small holder farmers and tribes throughout India. They carry out their programs through partnerships with other research institutions, private organizations, public organizations, and local communities that are affected directly. Project themes include, but aren't limited to: Food security, coastal systems research, biodiversity, biotechnology, ecotechnology, gender, and education dissemination. Solutions to problems identified at the center are researched in order for feasible solutions to be created. The foundation always communicates directly with the affected communities and farmers to ensure affective implementation of the solution.

2.2 Water4Crops Project

Many different industries exist within India. A majority of these industries create some amount of waste water, all being polluted in some way. The Water4Crops project is a five year long project which focuses on the bio-treatment of industrial wastewater for agriculture irrigation and aquaculture. Many NGOs throughout India have partnered on this important project to tackle the treatment of a wide array of wastewater types. MSSRF is specifically focusing on the distillery and sugar molasses industries.

The distillery and molasses industries create massive amounts of wastewater within their production. This wastewater is contaminated with melanoidins, carmels, phenols, and other harmful substances that pollute the waterways and environment. These substances found polluting the water make them unsafe for drinking or any other use that would be used for direct human consumption. MSSRF is focusing on bioremediation treatments for the wastewater as well as affective ways to implement constructed wetlands. If successful, this water can be used to help irrigate farmland on a consistent basis, as well as possibly implement aquaculture. MSSRF has partnered with KCP Sugar Industries Private Limited in Vuyyuru, Andra Pradesh for wastewater samples as well as solution implementation.

2.3 Justification

Water is becoming a scarce and important resource in many parts of the world, especially in India. Sadly, India is becoming the poster child for water pollution and scarcity problems. With a limited water supply and India's demand for drinking and irrigation water rising, alternatives need to be found to satisfy the needs of the country. Without alternatives, including treated wastewater, India will not be able to grow enough food to sustain a growing population in the future.

Using treated wastewater to irrigate crops would lessen the amount of potable water that is being used for agriculture; and therefore provide more drinking water to the growing population. Using treated wastewater to irrigate crops will also help ensure farmers that they will always have enough water to irrigate their crops. Recent droughts throughout India have lead to less clean and reliable water for farmers to use to irrigate their crops. However, many industries produce wastewater at a constant basis all year. If successfully treated, this wastewater can serve as a constant, reliable, and clean source of water for famers to use in surrounding areas of factories treating their water.

Water scarcity is a real problem that directly affects livelihoods and the amount of food that is produced for human consumption. The Water4Crops project keeps both environmentally sustainable practices and food security at the top of the list of requirements that must be met in order for the project to be considered successful. Bio-treatment solutions that are being researched are economically feasible and more appealing to industries. This will help with buy in from the industries. Without industry buy in, the project would be unsuccessful because no water would be treated, regardless of the research. The solutions being researched are also all environmentally friendly and help lessen the amount of pollution entering the waterways. Once successfully tested solutions are found, they will be put into place fully at KCP Sugar Industries Private Limited, and possibly at an array of other dominant molasses and sugar companies within India.

Upon arrival at MSSRF, I was given the freedom of choosing the project that I wanted to work in, granted that it coincided with my career interests. After graduating high school, I plan on studying environmental engineering. After hearing this, the Executive Director of MSSRF, Dr. Ajay Parida, suggested that I work on the Water4Crops project as it fits within my passions of food security and environmental sustainability. These goals set forth by Water4Crops project are both of the main goals that I have worked towards in the past and continue to be the main focus of what I want to research moving forward. For that reason, contributing to the Water4Crops project not only was beneficial to my education but also helped MSSRF in furthering its work.

2.4 Objectives of the Water4Crops project

Objectives of the Water4Crops project include:

- Identifying bacteria and fungus strains from farm land soil that effectively lessen the amount of phenols and melanoidins present in the wastewater to safe levels.
- Identifying bacteria and fungus strains from farm land soil that effectively discolors the wastewater making it clear and colorless.
- Lessen the amount of pathogens present, if any, in the wastewater to safe levels.
- Implementing constructed wetlands at the industries to treat the wastewater naturally in a cheap and energy efficient manner.
- Prove that bio-treated water is safe for agriculture irrigation and aquaculture.

Overall, if these goals are accomplished, the Water4Crops project will increase the amount of safe irrigation water that farmers can use to irrigate their crops.

2.5 Research Questions

Research questions that I focused on during my Borlaug-Ruan Internship include:

- What techniques can be used to identify bacteria strains in order to accomplish the project objectives?
- What water quality standards are currently in place nationally (in India) and internationally to help understand how polluted India's waterways are?
- How do constructed wetlands work?

2.6 Methodology and Limitations

Upon arriving at MSSRF, I dove right into my work within the Water4Crops project. I spent a few days reviewing past experiments conducted at different labs throughout India to better understand what I would be working on. I also worked for a few weeks with colleagues in my lab to better understand basic safety procedures as well as get a better understanding for why certain experiments and techniques were important for our project. All procedures and protocols that were used are ones used at the MSSRF microbiology lab on a regular basis. All concepts, like but not limited to why the project is important, how it functions, and why we conduct certain experiments, pertaining to the Water4Crops project were explained to me in conversation by my colleagues. I learned everything from basic safety procedures to follow in the lab to learning how to follow proper protocols for each experiment we would be conducting.

All protocols that were followed in the lab were adapted from *APHA 20th Addition, 1998*. These protocols are the protocols typically followed at MSSRF for scientific experimentation. Testing protocols used and adapted from *APHA 20th Addition, 1998* include: pH, total dissolved solids (TDS), electric conductivity (EC), dissolved oxygen (DO), chloride (Cl), organic nitrogen (N), phosphorus (P), and sulfate (S). These physicochemical property tests were conducted using three samples: Molasses Spent Wash (MSW), Anaerobic Treated Molasses Spent Wash (AnT MSW), and Tap Water- which simulated irrigation water.

Every test played a role in better understanding the composition of the wastewater. The pH test helped determine how basic or acidic the water was. The DO tests showed us how much oxygen was present in the water and therefore another indicator to as how well the water could support life. The TDS test helped us better understand how pure the water is and how much foreign matter was floating and dissolved within and throughout the water. EC was another indicator to has how pure and clean the water is. The more foreign matter that existed within the water, the more likely it was to be able to conduct electricity. The chloride, organic nitrogen, phosphorus and sulfate tests were all used to help determine exactly the composition of the foreign matter in the wastewater. By using the combination of the TDS, EC, and chemical composition tests we are able to understand the wastewater and its composition in a more precise way.

Other tests that were conducted or procedures that were followed during my internship at MSSRF include: polymerase chain reaction (PCR), DNA purification, DNA isolation, denaturing gel gradient electrophoresis (DGGE), decolourization, and phenol degradation. These tests were conducted using MSW and AnT MSW samples. DNA isolation, DNA purification, and PCR procedures were used also with samples from a farm test site. These three samples are denoted by NT1, NT2, and NT3. Each of these tests and/or procedures stated above is commonly used in all experiments conducted within the Microbiology lab at MSSRF, as well as other labs within the foundation. They are common practice throughout labs across the world.

PCR is a simple procedure which helps amplify DNA. The DNA being used is mixed with specific enzymes. The enzymes cut the DNA in certain areas, these DNA fragments are what are going to be amplified later. The fragments are then mixed with primers. The DNA is then loaded into a PCR machine which runs at different temperatures for different periods of time, depending on the fragment of DNA being amplified. Specific to our experiments, the temperatures and time periods are as follows: Initial denaturing at 95 degrees Celsius for 1 minute. Denaturing at 95 degrees Celsius for 5 minutes. Annealing at 60 degrees Celsius for 1 minute. Extension at 72 degrees Celsius for 1 minute. The denaturing, annealing and extension steps are then repeated for 35 cycles. Then final extension at 72 degrees Celsius for 10 minutes. Finally the samples are stored at 4 degrees Celsius until they are taken out of the machine. This PCR process that we followed is adapted from the Tabatabaei M *et al.,* 2010 protocol. The primer lends as a tool for the DNA to bind to in order for it to replicate many times. DNA isolation and purification are steps that take place before PCR. DNA is isolated either by serial dilution and then purified following steps developed by *APHA 20th Addition, 1998*. DNA purification was done by using a commercial purifying kit.

DGGE is a method used to study the microbial levels within a sample. DNA can be denatured two ways: either by heat or by a chemical. In DGGE, the DNA is denatured by a chemical. The gel for DGGE is cast within a specific range for the chemicals used (i.e. 30-80% or 40-50%) with the highest percentage on the bottom of the gel cast. Once the samples are loaded, the different DNA samples present travel through the gel until they are denatured by that specific chemical concentration. When a DNA sample gets denatured it creates a band showing that it stopped there. This method is useful when measuring microbial levels present in a sample, because each band represents a different microbe present. For example, a soil sample isn't going to only contain one type of microbe, but many types of microbes. When isolating the soil's DNA, the DNA isolated will contain the DNA for every microbe present. This helps us identify not only which microbes are present, but also which microbes are more prominent between different samples.

The decolourization experiment was conducted by using bacteria isolates already identified by scholars at MSSRF as being effective in lessening the amount of color present in wastewater. Bacteria isolates used include: MSSRF W20, MSSRF H1.1, MSSRF H36, P. putida, and a consortia mix which included equal amounts of all four isolates named above. Wastewater samples were measured every 12 hours for a total time period of 60 hours to determine their effects of lessening the water's color.

The phenol degradation experiment used the same bacteria isolates as the decolourization experiment. The medium used in this experiment was composed of: Glucose (carbon source)- 1%, KH2PO4- 0.2%, K2HPO4- 0.1%, MgSO4- 0.05%, Yeast Extract (Nitrogen Source)- 0.1%, and Phenol stock solution, 200mg/L. For inoculation, 5 ml of the bacteria isolate was inoculated in 100 ml of the wastewater medium in a 250 ml Erlenmeyer flask. For the consortia mix, 2 ml of each bacteria isolate was inoculated into the medium. Two replications were made of each sample. The samples were stored at room temperature and were shook 24 hours a day except for when being tested. Samples were tested until a consistent reading was observed, confirming that there was no further phenol degradation within the samples.

Each test individually does not have the capability to definitely determine and deem the wastewater to be safe or unsafe for agriculture irrigation. However, the combination of all of the tests allows us to look at many different layers and complexities of the water and better help determine how safe it is. All data collected from tests that followed the *APHA 20th Addition, 1998* protocol were compared to international standards set forth by the FAO and ICRISAT. The FAO is a branch of the United Nations and ICRISAT is an agriculture NGO in India working on the Water4Crops project. Both organizations have released standards for which they deem water would be safe to use for irrigation.

Lastly, I briefly studied constructed wetlands and how the affective use of constructed wetlands can help. Constructed wetlands are a natural way of treating wastewater. Water travels through different sized gravels, sands, and through areas with plants. These different areas naturally take the microbes polluting the water out. Engineers and scholars at MSSRF designed and constructed a wetland at KCP Sugar Industries Private Limited in Vuyyuru, Andra Pradesh, India.

There were a few limitations that existed within my work on the Water4Crops project. First, the wastewater samples that I was working with were collected approximately a month prior to my arrival at MSSRF. This time period could have allowed for ph, TDS, EC, DO, P, Cl, and S levels to change. Another limitation that I encountered was the equipment that was required for the Nitrogen test. We did not have the proper glass ware on hand for that particular experiment. We tried distilling the wastewater anyways, but the results showed that there was no nitrogen present in the water. Using deductive reasoning, we knew that there had to be at least trace amounts of nitrogen present in the water and therefore those results were inconclusive. But we had no way, at the time, of effectively determining the nitrogen levels in the wastewater.

III. Background of People in Project

While studying at MSSRF I worked within the Microbiology department. I worked in the lab on a day to day basis. The Microbiology lab at MSSRF is lead by Dr. V.R. Prabavathy. Under Dr. Prabavathy's supervision and leadership I worked with the PhD scholars within the lab. I mainly worked with Purushothaman Duraisamy every day on my experiments.

Purushothaman is a PhD thesis scholar who is doing his PhD studies within the Water4Crops project. He came to MSSRF for the first time to complete a research project to earn his Undergraduate degree. Afterwards he went on to complete his Masters degree, again coming back to MSSRF to complete his research project to complete his Masters degree. After his Master's project was complete, he was offered a spot at MSSRF as a PhD scholar. He spent the first year of his PhD studies working on a bio-fertilization project. However, due to problems that arose within the project, he was reassigned to the then newly starting Water4Crops project. He has been working on the Water4Crops project since the beginning, and is using this as the sole research for his PhD work. Purushothaman helped me every day with my experiments and taught me overall about the Water4Crops project and what it encompasses. My understanding of the project came from the conversations and experiments that I conducted with him.

Although I worked with Purushothaman on a daily basis, I worked with other MSSRF employees and PhD scholars throughout my stay as well. Other people that I worked with at MSSRF during my internship include: Justin, Ganga, Baskaran, Jegan, Kathiravan, and Shanthakumar. Justin is an employee at MSSRF's field site at KCP Sugar Industries Private Limited in Andra Pradesh. He showed me the constructed wetlands during my field visit and helped teach me more about constructed wetlands and their importance. Ganga is a PhD scholar who taught me the basic lab procedures and safety protocols to follow when I first arrived at MSSRF. Baskaran is a PhD scholar who taught me about DGGE and the protocols to follow for the experiment as well as why DGGE is an important experiment to use. Kathiravan is a PhD scholar who taught me about PCR and why it is an important technique to use in many experiments. Jegan and Shanthakumar are both senior fellows at the Microbiology lab. They both recently received their PhDs. Both men helped me better understand lab techniques and the data results.

Even though only a few names are listed above, many other people at MSSRF helped me with my research throughout my stay at MSSRF. A couple different departments at MSSRF work on the Water4Crops project. Most work is done within the lab with successful solutions being implemented at the field site at KCP Sugar Industries Private Limited in Andra Pradesh.

IV. Contributions and Responsibilities

4.1 Tests Conducted and Procedures Followed

Once it was decided that I would be working within the Water4Crops project, my supervisor Dr. V.R. Prabavathy outlined my research plan on what experiments I would be conducting. From discussions between Dr. Prabavathy, Purushothaman, and me it was decided that I would be conducting the following physicochemical property experiments on the wastewater samples: pH, Electric Conductivity (EC), Total Dissolved Solids (TDS), Dissolved Oxygen (DO), Phosphorus (P), Chloride (Cl), Sulfate (S), and Nitrogen (N) testing. It was also decided that I would learn specific advanced techniques in the lab including: PCR, DNA isolation and purification, and denaturing gel gradient electrophoresis. Lastly, we decided that I would also conduct decolourization and phenol degradation tests.

4.2 Results Further Usage within Water4Crops Project

The physicochemical property tests that I conducted on the water samples and the results that were collected are original to me and my own work. However, those results will not be used in the Water4Crops project as those tests were already collected immediately following the water's collection. I conducted the physicochemical property tests to better understand the project as well as learn the protocols that were needed to carry out each test. The PCR, DNA isolation and purification, and denaturing gel gradient electrophoresis procedures that I followed and conducted on the wastewater samples and the results that I collected are original to me and my own work. However, those results and procedures were used to help further the Water4Crops project. The DNA isolation, purification, and PCR that I did were all used to further experiments within the Water4Crops project. The denaturing gel gradient electrophoresis, decolourization, and phenol degradation results that were collected were used by my colleagues to help better understand the project and move forward with further experiments after I left MSSRF.

V. Results

5.1 Physicochemical Properties

Water pH:

Sample:	Value:
AnT MSW	7.6
MSW	4.48
Tap	8.01

Organization:	Guideline:
FAO	6.5-8.4
ICRISAT	6.5-8.4

Water TDS:

Sample:	Value (mg/L):
AnT MSW	19.885
MSW	12.34
Tap	2.30

Organization:	No restriction on use (mg/L):	Moderate restriction on use (mg/L):	Severe restriction on use (mg/L):
FAO	<450	450-2000	>2000
ICRISAT	<450	450-2000	>2000

Water EC:

Sample:	Value (dS/m):
AnT MSW	77.32
MSW	37.85
Tap	7.13

Organization	No restriction on use (dS/m):	Moderate restriction on use (dS/m):	Severe restriction on use (dS/m):
FAO	<0.7	0.7-3.0	>3.0
ICRISAT	<0.7	0.7-3.0	>3.0

Water DO:

Sample:	Value (mg/L):
AnT MSW, 99.9% diluted	1.8
MSW, 99.9% diluted	1.4
Tap, 99% diluted	3.1

There are no international or national standards available for Water DO. Therefore no comparison was made.

Water Phosphate:

Sample:	Value (mg/L):
AnT MSW	1,732.6
MSW	1,527.5
Tap	174.3

There are no international or national standards available for Water Phosphate. Therefore no comparison was made.

Water Chloride:

Sample:	Value (meq/L):
AnT MSW	648.1073
MSW	465.96
Tap	19.767

Organization:	No restriction on use (meq/L):	Moderate restriction on use (meq/L):	Severe restriction on use (meq/L):
FAO	<4	4-10	>10
ICRISAT	<4	4-10	>10

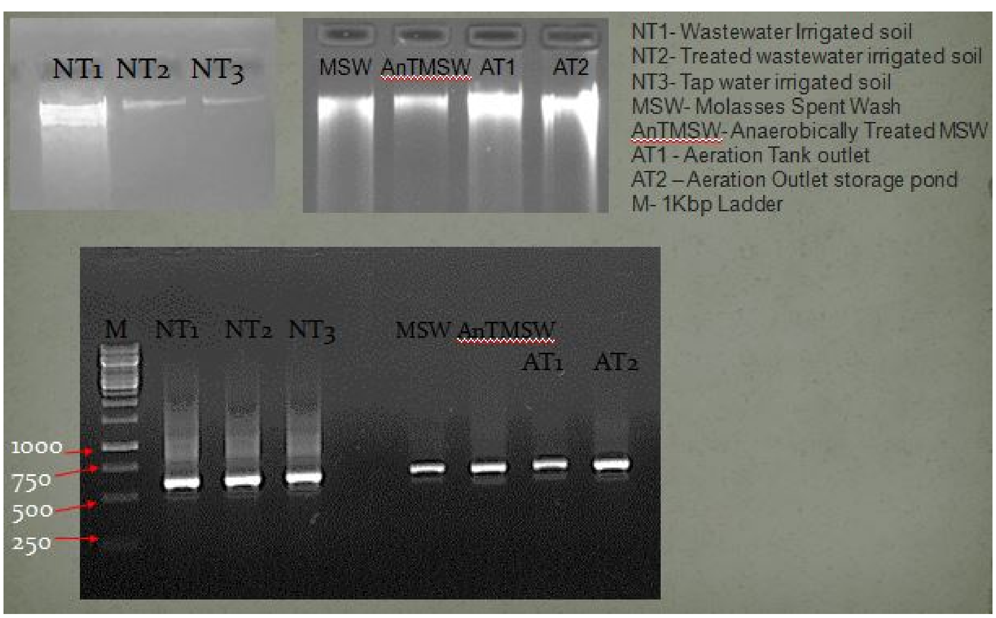
Water Sulfate:

Sample:	Value (mg/L):
AnT MSW	46,630
MSW	58,850
Tap	12,704

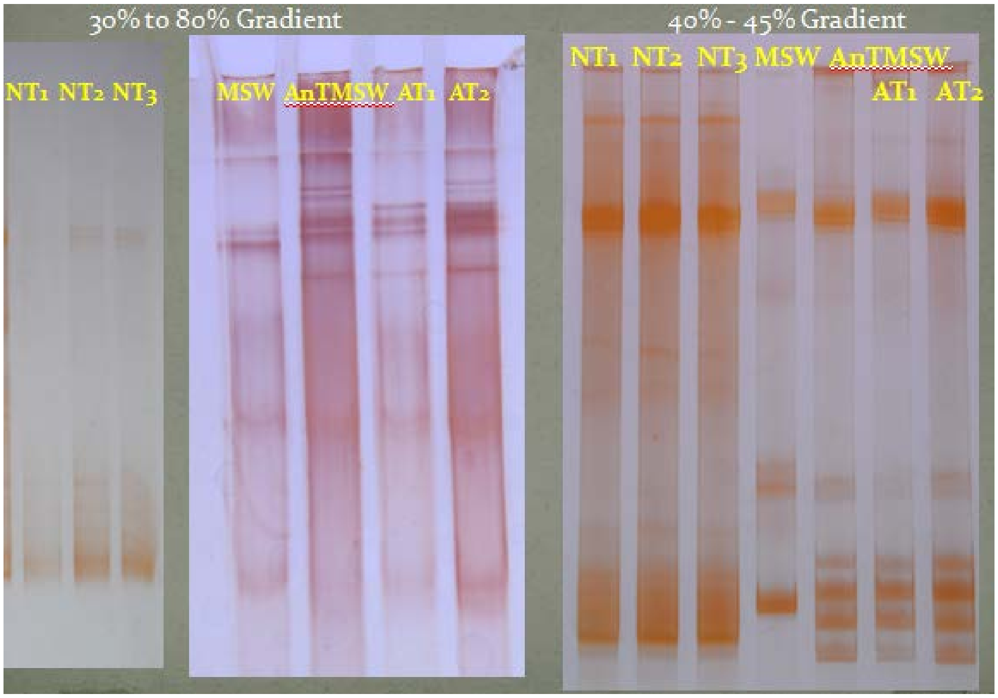
There are no international or national standards available for Water Sulfate. Therefore no comparison was made.

5.2 DNA Isolation and Purification

Note: PCR was one of many techniques used to accomplish these results.

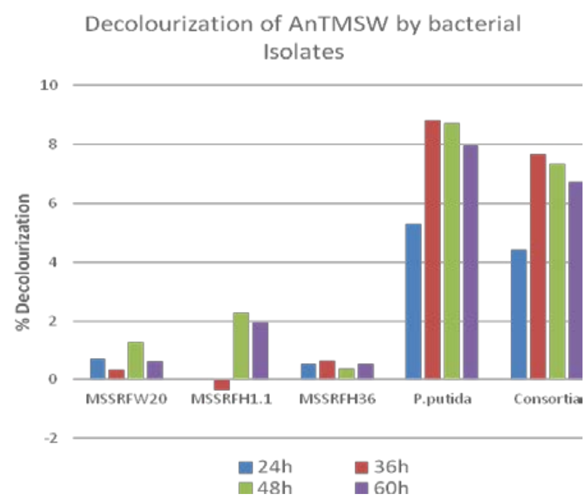


5.3 Denaturing Gel Gradient Electrophoresis



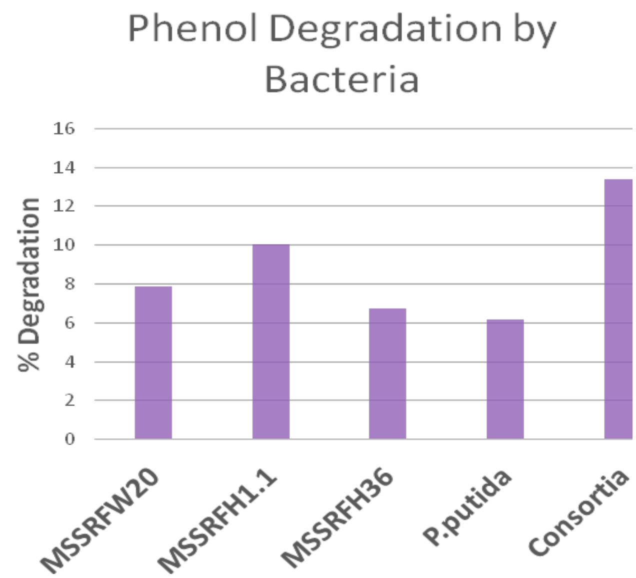
5.4 Decolourization Effect of Bacteria Isolates

	% of Decolourization			
Bacteria	24h	36h	48h	60h
MSSRFW20	0.710985	0.343761	1.265823	0.617284
MSSRFH1.1	0.035549	-0.34376	2.285513	1.960784
MSSRFH36	0.533239	0.653145	0.386779	0.544662
P.putida	5.296836	8.834651	8.720113	7.95207
Consortia	4.408105	7.665865	7.348805	6.717502



5.5 Phenol Degradation by Bacteria Isolates

Bacteria	6th day
MSSRFW20	7.899%
MSSRFH1.1	10.052%
MSSRFH36	6.763%
P.putida	6.161%
Consortia	13.365%



VI. Conclusion and Impact

6.1 Physicochemical Properties

Sample:	pH:	TDS:	EC:	DO:	P:	Cl:	S:
AnT MSW	Yes	Yes	No	---	---	No	---
MSW	No	Yes	No	---	---	No	---
Tap	Yes	Yes	No	---	---	No	---

The table above shows which physicochemical tests had international and national standards provided to compare our results to; it also shows which tests meet safety standards based off of comparisons made with the international and national standards (FAO and ICRISAT). Anaerobic treated molasses spent wash is AnT MSW. Molasses spent Wash is MSW. Tap water simulated agriculture irrigation water since I didn't have access to irrigation water from a farm at the time of my stay at MSSRF.

Interpretations for each physicochemical test are as follows:

PH: AnT MSW: Falls within pH range. By this standard, is suitable as irrigation water. MSW: Falls outside pH range. By this standard, isn't suitable as irrigation water. Tap: Falls within pH range. By this standard, is suitable as irrigation water.

TDS: By this standard, all three samples could be used for irrigation.

EC: None of the samples meet the EC standard and therefore aren't suitable for irrigation based off of this standard.

DO: Tap water is 10 times less diluted and still has a DO level about double that of the wastewater. Tap water levels are high enough that if in a stream, fish and other organisms could survive. There are no international or national guidelines set concerning DO levels for irrigation water. Therefore, we can't draw conclusions of irrigation water suitability based off of this test.

Phosphate: Wastewater contains about 10 times more phosphate than the tap water. There are no international or national guidelines set concerning phosphate levels for irrigation water. Therefore, we can't draw conclusions of irrigation water suitability based off of this test.

Chloride: Both wastewater samples have levels too high for irrigation. Tap has a level double the amount allowed for surface irrigation.

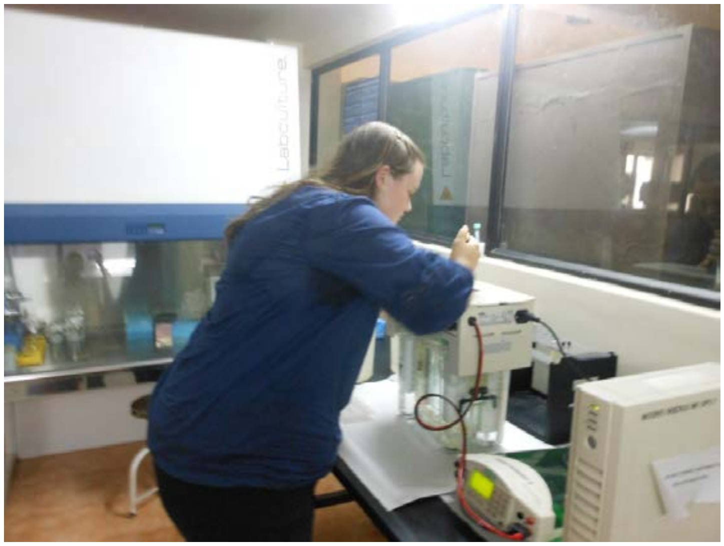
Sulfate: Wastewater samples contain roughly 4-5 times more sulfate than the tap water samples. There are no international or national guidelines set concerning sulfate levels for irrigation water. Therefore, we can't draw conclusions of irrigation water suitability based off of this test.

Organic Nitrogen: This test was attempted several times. Due to improper glass ware availability in the lab, we were not able to properly distill the wastewater for the test to follow proper protocol. For that reason, our results were inconclusive.

Based off of the interpretations made on the physicochemical tests, AnT MSW and Tap water seem to have the same suitability for agriculture irrigation water. From deductive reasoning and common sense we know that is simply not true. We can physically see from the color of the water that the wastewater is not suitable for agriculture irrigation. Therefore, further tests are needed to fully understand the composition of the industrial wastewater from molasses and sugar industries. Without knowing the full composition of the wastewater there is no way of knowing whether or not the wastewater is suitable for agriculture irrigation.

6.2 DNA Isolation, Purification, and DGGE

After several attempts DNA isolation and purification from the soil samples and wastewater samples were successful. This purified DNA was then used in the DGGE experiment several times. The DGGE experiment was completed within my last week of studies at MSSRF. After leaving MSSRF, my colleagues plan on then cutting the bands apart from the DGGE gel to then individually identify each microbe that is present in the samples that were present in the soil and wastewater samples. Once gaining the understanding about all of the microbes present in our samples, the Water4Crops project will be able to move forward in identifying ways and organisms that could successfully treat the wastewater.



Me loading the samples into the chamber for DGGE

6.3 Decolourization Effect of Bacteria Isolates

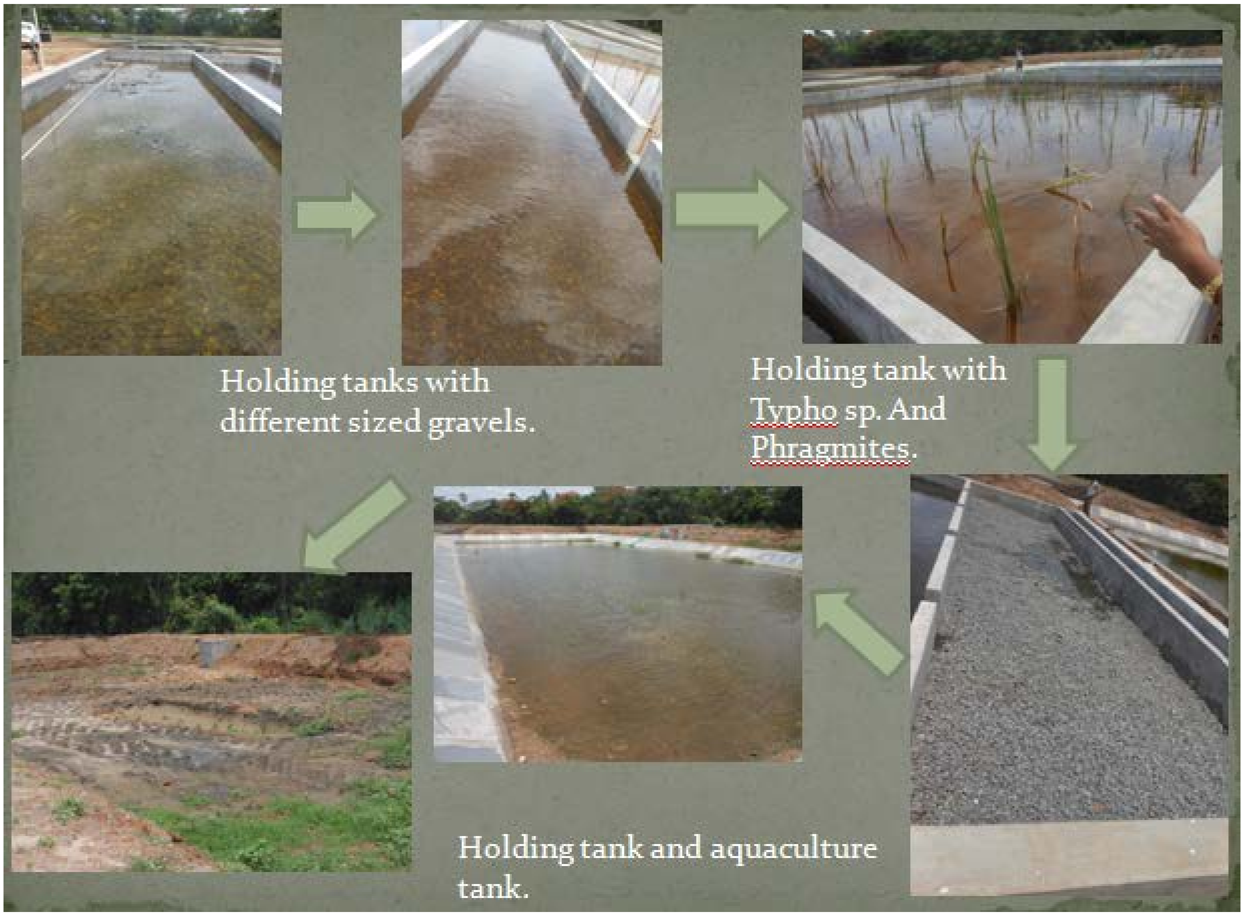
The bacteria isolate P. putida proved to have the highest percentage (~7.9%) of lessening the amount of color present in the wastewater samples. The consortia mix had the second highest rate (~6.7%) of lessening the amount of color present in the wastewater samples. Though this is still not a high percentage, it's a step in the right direction. These strains were already identified by MSSRF (by isolation from soil samples) as having potential to lessen the color of wastewater. With further research, there is the possibility to find another bacteria or stronger ratio for the consortia mix to lessen the color of the wastewater. Lessening the color in the wastewater is important because it visually shows that there is less foreign matter within the wastewater present. It also gives more buy-in for the farmers to use the treated wastewater for irrigation because they can see that it is clean and safe for food production.

6.4 Phenol Degradation by Bacteria Isolates

The consortia mix had the highest phenol degradation (13.365%) of the wastewater samples. The bacteria isolate MSSRF H1.1 had the second highest phenol degradation (10.052%) of the wastewater samples. Even though these rates aren't that high when considering that the Water4Crops project has a goal to completely get rid of phenol in the wastewater, it is a step in the right direction. MSSRF already identified these bacteria isolates (by isolation from soil samples) as being possible isolates to successfully degrade phenol completely from the wastewater. With further research there is the possibility of finding a bacteria isolate or new consortia mix that completely degrades the phenol from the wastewater.

6.5 Constructed Wetlands

From by field visit at KCP Sugar Industries Private Limited in Andra Pradesh, India and my discussions with my colleagues at MSSRF I learned an immense amount of knowledge about constructed wetlands and their importance to treating industrial wastewater in a low-energy environmentally friendly manner.



Constructed Wetland Cycle

The cycle that the wastewater follows to be treated by the constructed wetland is very energy and cost efficient. First anaerobic treated wastewater is pumped into the first holding tank. The water than slowly travels through the first holding tank that contains big rocks, to another holding tank, which holds smaller sized stones. The water travels from tank to tank all by gravity, so no electricity is needed. The water then flows into a holding tank containing Typho sp. and Phragmite plants. These plants have been identified to grow in the wastewater while pulling contaminants out of the water through their growth. After that the water flows into one more tank containing really small pebbles. Once through this tank it flows into a tank where it sits for two weeks. Once completing that stage it will flow into an aquaculture tank where it will stay for one year with fish.

Construction of this constructed wetland at KCP Sugar Industries Private Limited, Andra Pradesh, India was completed in June of 2014. In September of 2014, after I had already arrived back to the U.S., the MSSRF team was planning on pumping the water from the last holding tank into the aquaculture tank and adding fish. After a year, the team will test the water and the fish produced from the aquaculture ecosystem to see how well the constructed wetland treated the wastewater. Preliminary tests are showing that the constructed wetland is successful at treating the wastewater.

Some observations that I had from visiting the constructed wetland include: It is proving to be a viable way of treating water. When the water entered the treatment cycle it was very brown in color, but in the end of the cycle I could see a notable difference in water quality. The water was almost completely clear. The water moves effectively, with low energy usage. There is no floor bottom in final tank. This is because there is not a problem or risk of leaching. Aquaculture also plays an important role in the constructed wetland cycle. If proven safe in the field, the aquaculture will not only help clean the water completely but will also produce fish as another food source for people nearby to constructed wetland. Lastly, in the last small gravel tank it seemed as though the water wasn't being moved easily as there was very little water present within the tank. However, it was explained to me by maintenance staff at the field site that there was a problem with construction which inhibited the complete flow of water from that once tank to the next. And that the problem was being corrected in the coming months.

6.6 Final Conclusion on all Aspects of Project

I learned a lot about wastewater and how it can play an important role in increasing safe food production in India since farmers would have a source of clean water for irrigation. Since the Water4Crops project is a five year long project, I only was able to see and help in a small part of the project as I was only at MSSRF for two months. Through all of my experiments I was able to conclude that further testing is needed to fully understand the composition of the wastewater. Once the composition is fully understood, viable solutions will be able to be found to treat the wastewater. Once a bio-treatment is found completely successful, farmers throughout India will have a constant, clean, and reliable source for irrigation water. This will greatly influence food security throughout India as water is becoming scarce throughout many regions.

Overall, the Water4Crops project will help improve food security greatly in the long run. The tests that I helped complete were carried out for two reasons. A.) So I could have a better understanding of the methodology used on a day to day basis in the lab for careers associated with this work, and b.) to help in the research of this project with producing some data. My contribution to this project is small. However, this small contribution will help in the continuance of MSSRF's groundbreaking research with the Water4Crops project.

The Water4Crops project will help improve food security in many aspects. They include, but are not limited to: Helping produce more food. This is due to more water being available for more planting; as well as helping continue to grow food in areas that it isn't always feasible because of recent droughts. Lessening the demand of potable water. Using reclaimed treated wastewater effluent will allow for less potable water to be used for crop irrigation. Overall this means that more potable water will be available for drinking water purposes. Helping farmers come above and/or stay above the poverty line. By providing a more reliable source of water (the farmers can trust that this water will most likely be available on a constant basis since industry is using water on a constant basis) farmers are able to grow their crops in all allowable seasons regardless of rain. As well, they are less likely to lose their crop yields due to droughts. Having more reliable growing seasons as well as possibly increasing the amount of growing seasons means that farmers can produce more food. Ultimately, earning more money for their family. This extra hard earned money will benefit the families in multiple ways, keeping them above the poverty line. The work that I helped MSSRF in and that they continue will eventually lead to an increase in safe food production across India. I feel confident that the combination of bioremediation and the use of constructed wetlands will lead to industrial wastewater in the molasses and distillery industries to be completely treated and safe for agriculture irrigation.

VII. Personal Reflection

Writing this only a month after returning from India, it's still hard to fully comprehend the difference in culture and what I experienced. I appreciate simple things more in my everyday life like a warm shower or having toilet paper. I showered in cold water almost every day, only having hot water available a few times. I carried toilet paper with me since it wasn't available anywhere that I went to the bathroom outside of my room. With that, I now appreciate western toilets immensely. I didn't realize how such small things become big luxuries in other parts of the world. I've become a lot more peaceful in all aspects of my life since returning from India.

I've learned how much less of an importance we should place on material possessions and the gift of sharing. In the Indian culture, one should only really have what is necessary to complete their work. Most people live very simple lives only buying something if it is a necessity or their old one completely broke, never to update the old. People share everything, rarely being greedy. I remember driving through Chennai with my supervisor during my first week in India. I was astonished at how many fruit stands are on the side of the road. I asked her if people hauled these huge heavy stands everyday or if they just took the fruit home with them, leaving the stand behind. She explained that many farmers leave the stand behind, only taking the fruit with them. They don't have to worry about theft or vandalism, even in a large city like Chennai, because there is a common sense of community. A person who doesn't know the fruit vendor, but sees her selling fruit every day, will feel obligated to protect the stand if he sees somebody messing with it, as she is a part of the community. Since everybody feels obligated to help others, there is very little vandalism or theft. I find myself greatly missing that sense of community now back in the U.S.

I think one of the best representations of the Indian culture is within their food and the customs that follow it. First, I miss traditional southern Indian food so much. The chutnees, sambars, rice, and other traditional dishes have not been fully replicated in my kitchen at home. Not only is Indian food spicy and delicious it is another reflection of how close a community is. At the lunch table at MSSRF, everybody opened their canteens or got food from the foundation's kitchen. But once at the table, a person's food was open for consumption by any other person at the table. Everybody always traded with one another and ate with their hands. At the start of my meal I had 3-5 curries and chutnees, but the end of my meal I had consumed close to 20 different types as all of my friends had shared with me. This sense of community is unparallel back in the U.S. A person's family isn't just your blood family but the people who you interact with on a regular basis.

In retrospect, I've learned truly how privileged I am to live in the U.S. and the overconsumption that takes place on a regular basis within our culture. I've realized from seeing food insecurity problems first hand on a daily basis that food insecurity is a multifaceted problem. There isn't one solution that will solve everything. Because of that, it will take many people to overcome food insecurity but it is possible. Something as simple as water contributes a huge factor into the food security problems that people face.

I've seen firsthand that when people go with less, they are more appreciative of what they do have. Food is equivalent to hard work. Getting food in most parts of the world, including India, takes more effort than going to the grocery store. It takes a lot of back breaking work, of which people aren't awarded for properly; because many times they are still living in poverty.

I had the opportunity to attend the Asia Pacific Regional Consultation: Role of Family Farming in the 21st Century Conference while at MSSRF. This conference opened my eyes to both a breadth and depth of prospectives and problems facing food security throughout the world during the four day span that the conference was held. My perspectives have completely changed. My definition of food insecurity used to simply be a battle between organic and conventional farming practices. My perspectives of the different variables included in the food insecurity problem have transformed or even changed completely. I've been completely humbled by what I've experienced and for that I'm extremely grateful. I feel empowered and inspired to continue this fight.

VIII. Works Cited

All protocols and procedures followed for experiments were cited earlier in methodology. All background information pertaining to the Water4Crops project was explained to me in conversation by my colleagues at MSSRF. All work, results, pictures, and graphs included are original to me and my own work.

IX. Pictures from Project

Isolating DNA

Loading Electrophoresis Gel

Purifying DNA

Conducting Dissolved Oxygen Test

Constructed Wetland Visit

Conducting Phenol Degradation Test