Clawson-Youngs Reports 2023:
Environmental Studies Through Various Lenses

Reports Authored by Clawson-Youngs 2023 Recipients

Editors: Dr. J. William T. Youngs and Elena McKinley

Introduction: Elena McKinley

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Foreword

Several years ago, Alicia Kinne-Clawson and Michael Clawson kindly created The Bill Youngs Endowment at Eastern Washington University. The first project under the endowment is known as the Clawson-Youngs Environmental Studies Award. To qualify undergraduate and graduate students in any field on campus submit a proposal for research on a theme in environmental studies. This booklet consists of the reports written by each of the students on their project results. The introduction that follows was written by Elena McKinley and provides a brief overview of each of the subjects.

- J. William T. "Bill" Youngs

Introduction

The 2023 Clawson-Youngs topics cover an array of subjects which pertain to our environment. The reports vary in scope but there is a through-line that links each of these environmental studies papers together – well-researched, thoughtful care delving into an area of the natural world.

Jonah Frago's "Development of Competency Protocols for the Honey Bee Gut Symbiont, *Snodgrassella alvi"* dissects the potential of introducing new DNA into honey bees. DNA manipulation for the purpose of affecting the immune system of these bees offers insight into engineering S. alvi. and what that scientific advancement could mean.

"The Future of Skiing and Snowpack in Spokane" by Elizabeth Graves is an exploration into how climate change is affecting snowfall in Spokane, Washington. She draws the correlation between our changing environment's effect on the local skiing community – and the ways that change impacts Spokane's tourism and overall community health.

In "Federal Courts, a Native Tribe, and Reviving a Lake" Aaron Hedge discusses the efforts of the Coeur d'Alene Tribe of North Idaho to (re)gain control of the lake bearing their name. The tribes base their claim on the historic ownership of the lake and the argument that their philosophy of water management is more conducive to the health of the lake than that of the state of Idaho. The tribe considers the long-term effects on future generations, while the state focuses more on economic growth.

"The Restoration of Species on the Palouse Prairie" by Thurman Johnson plants the seeds of optimism for achieving flora resurgence on the Palouse Prairie. The report examines factors such as topography and diversity of seed composition as well as the importance of revitalizing the region's native plant growth.

Devin Mumey's "Lichens of Iller Creek" explores the Dishman Hills Conservation Area – specifically, Iller Creek – to survey and identify varying species of lichen. A better pathway to lichen identification will provide further understanding of a complex lifeform as well as the creek's diverse ecosystem.

Claire Richardson's "Elemental Concentrations in Urban and Rural Dust in Spokane, Washington" examines the concentration of heavy metals found in urban versus rural air. The study compares and contrasts levels in the city of Spokane and in Turnbull National Wildlife Refuge in pursuit to better map the area's complicated air quality.

"Modeling Groundwater Toxin Flow in the West Plains" from Erin Toulou dives under the land in the West Plains to gauge the water pathways of toxins that may be lurking below the surface. The Airway Heights section of the area has been the subject of water contamination in recent years – posing a potential public health hazard.

- Elena McKinley

Jonah Frago
Mentor: Dr. Jenifer Walke

Development of Competency Protocols for the Honey Bee Gut Symbiont, *Snodgrassella alvi*

Abstract:

Western honey bees, *Apis mellifera*, are crucial pollinators for agriculture and ecosystems, but are often affected by various diseases. One potential solution is to engineer the gut bacteria of honey bees to produce pro-immune response metabolites. *Snodgrassella alvi*, a core bacterium in the honey bee gut and member of the *Neisseriaceae* family, has been identified as a promising candidate for engineering, as it has been shown to effectively control certain health issues such as Varroa mite infections activating the hosts RNAi defense system through dsRNA production. This research is extremely promising, however the study relied on conjugation to transform the bacteria due to a lack of protocols to induce competency in *S. alvi*. Through protocols for taxonomically similar microbes, I will induce competency in *S. alvi*, allowing the introduction of exogenous DNA. Success in this project will allow future researchers a much simpler, less expensive approach to engineering *S. alvi*.

Background:

Molecular Engineering:

Molecular engineering microbes revolves around the process of introducing new DNA into a bacterial cell. This DNA can be designed by scientists or taken directly from other natural sources, but either way, it exists as a plasmid, a small circular piece of DNA that stays inside the cell but exists outside the host genome. In nature, plasmids are produced from the remnants of dead cells' genomes and are generally taken up by current generations in the face of harsh environments or extreme changes to adapt to new conditions. A cell able to take DNA from its environment is called competent, and a competent cell taking DNA from its environment is referred to as transformation. As scientists looking to implement specific DNA into a microbe, we exploit this evolutionary adaptation by exposing the cells to various extreme conditions to artificially induce competency, then introduce our DNA of interest for the microbe to uptake. There are several methods for inducing competency, in this project, I focus on Chemical, and Electro-competency.

Chemical and Electro-competency:

These two states have similar conditions for induction; however, they differ in the method required to implement foreign DNA. Both processes use chemical washes to make the cell membrane more permeable to DNA molecules. Additionally, keeping the cells extremely cold is also vital for success in inducing competency as we must keep the cells in a stressed state. After several rounds of chemical washes, and centrifugation, the cells should be made competent and then can either be used immediately or stored at -80°C.

Heat shock and electroporation transformation:

Once competent, depending on the method used, cells may be transformed by either heat shock for chemically competent cells, or electroporation for electrocompetent cells. Heat shock transformation relies on extreme temperature changes as a final stressor for the cells, increasing their rate of extracellular DNA uptake. Electroporation uses a machine called an electroporator which generates a strong electric field across the cells to create pores in the plasma membrane allowing DNA to be taken up. Both methods have strengths and weaknesses and different efficiencies in different bacterial species. However, electroporation is much more difficult to create protocols due to the variability in electrocution settings.

Supporting research:

The primary research that inspired this project was a paper published in 2020 by Leonard, et al. called "Engineered symbionts activate honey bee immunity and limit pathogens". This study showed that researchers could implement DNA into *S. alvi* which would produce dsRNA which acts as a primer in insect immune systems. The introduced engineered symbionts showed a significant decrease in the lifespan of the mites feeding on the honey bees. They also discuss the possibility of introducing RNAi genes designed to interfere directly with specific pathogens. However, because of the lack of competency induction protocols, the researchers relied on the costly, time-consuming process of conjugation, thus the goal of my research was to develop these induction protocols making the process of introducing beneficial DNA into *S. alvi* much easier.

Methods:

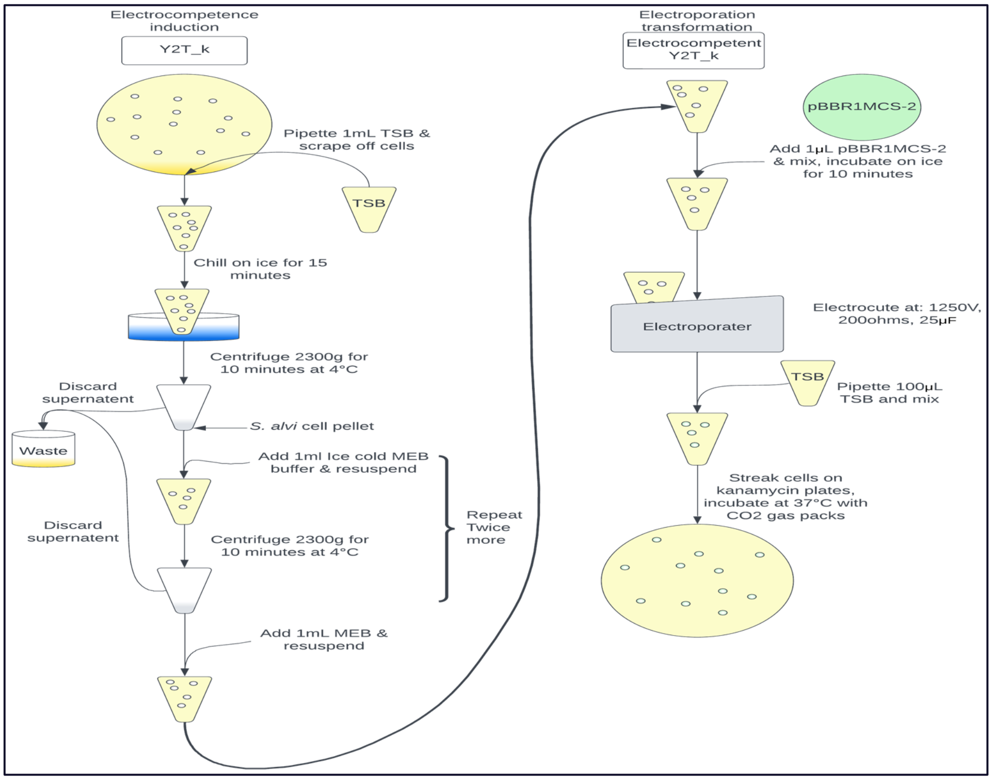
Plasmid selection:

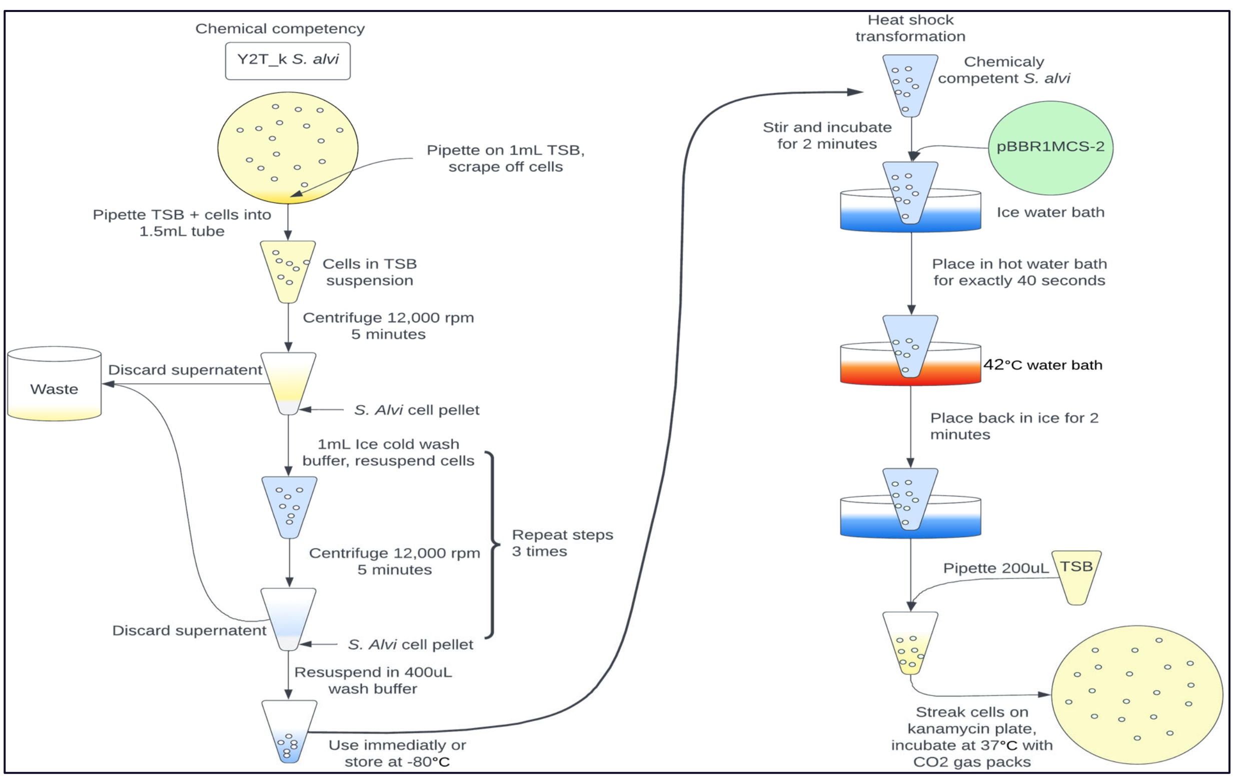
Plasmid selection is an important first step in molecular engineering. You must choose a plasmid with the features you need, such as resistance to a specific antibiotic or fluorescent marker proteins. I chose the plasmid pBBR1MCS-2 for its wide host range, kanamycin resistance gene, and lac operon, which can be induced to turn transformed colonies blue for additional confirmation of transformation success.

Development of protocols:

I developed my protocols for chemical and electro-competency by adapting existing protocols from the book "Electroporation Protocols for Microorganisms".

The following diagrams represent the two protocols I developed and tested.

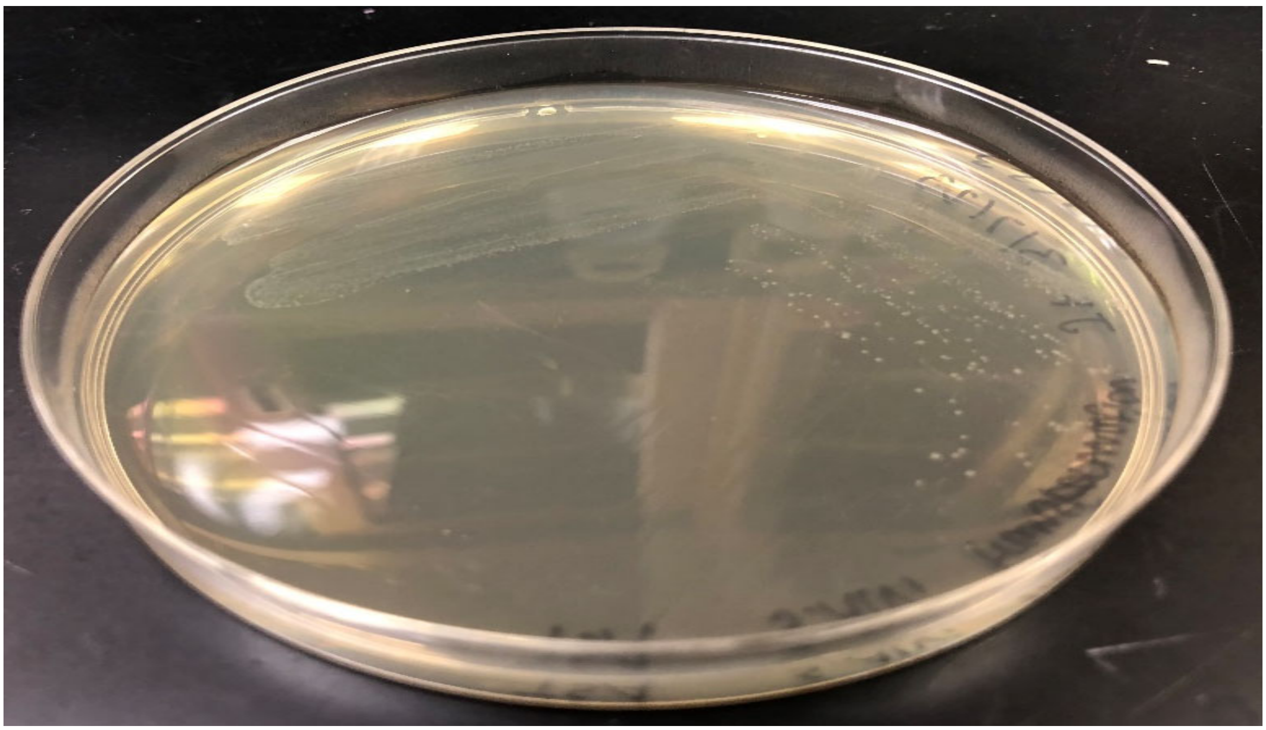




The variables I adjusted to develop these protocols were time in a hot water bath, the number of washes for chemical competence, and voltage and resistance for electro-competence and transformation.

Results:

After several rounds of attempts, I was only able to achieve one successful transformation. I was not able to achieve any successful transformations using the electro-competency and electroporation transformation protocol. Due to the degree of adjustability in the electroporator settings, knowing how to calibrate proper voltage, resistance, and capacitance to a novel bacterial species is difficult. However, using the above chemical competency and heat shock protocol, I was able to transform *S. alvi* with the pBBR1MCS-2 plasmid. This result is shown by the growth of *S. alvi* colonies on a kanamycin plate in the image below. Without successful transformation, the antibiotic would prevent any bacteria from growing. Here we can see the small white colonies of transformed *S. alvi*. The colonies were smaller than typical *S. alvi* growth, this is likely due to the presence of kanamycin which may still be impeding growth despite the antibiotic resistance gene.



The growth of S. alvi colonies on a kanamycin plate after transforming S. alvi with pBBR1MCS-2 plasmid.

Future research:

I would like to continue this research by examining any differences in transformation efficiency across various strains of *S. alvi*. I was only able to use one strain of *S. alvi* for this research due to time constraints, but the next step of this project will be repeating the chemical competency and heat shock transformation protocols across six to eight different strains and analyzing if any strains in particular are best suited for engineering.

Acknowledgements

We thank Macee Mitchell (EWU) for providing bacterial cultures from honey bees and our collaborators at Virginia Tech (Drs. Lisa Belden, Richard Fell, David Haak, and Emma Bradford) for providing bees. This work was funded by the National Science Foundation MCB-SSB Award #1817717 to JBW, as well as the Clawson-Youngs Environmental Studies Award.

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Elizabeth Graves
Mentor: Rachel Toor

The Future of Skiing and Snowpack in Spokane

The 2022/2023 season was my first winter in Spokane. I live in the University District, and the city's first big snowstorm happened on November 30th. Eastern Washington University called a snow day, so I was working from my apartment when I looked up to see an old white Subaru pulling a skier on a tow rope around my block. Half an hour later, a Nordic skier skate-skied down the middle of the street. From then on, all afternoon an assortment of cars pulled an ongoing parade of skiers, snowboarders, and sledders down streets. That was my first real indication of Spokane's ski town nature, and I was overjoyed to know that come first snow, Spokane residents pull their gear out of closets and take to the streets. It felt like a promising sign for a good season to come.

There are five ski areas (Mount Spokane Ski & Snowboard Park, Silver Mountain Resort, Lookout Pass Ski & Recreation Area, 49 Degrees North Mountain Resort, and Schweitzer Mountain) within a 90-minute driving radius from Spokane, along with many backcountry skiing opportunities, so it's no wonder that there is such a vibrant subculture of skiers living in Spokane. Unfortunately, it's also no surprise that climate predictions warn of an uncertain future for the ski industry, with warming temperatures, reduced snowfall, and shortened seasons. Beyond the threat of losing a recreational activity and lively subculture, the ski industry in Spokane attracts tourism (the Spokane Airport is a hub for skiers to access all five ski areas and the mountains of interior British Columbia, Canada) and creates seasonal and year-round employment opportunities for people living in Spokane. Snowpack also provides a valuable water source that fills rivers and reservoirs, replenishes groundwater reserves, and directly affects the amount of water available for the entire year. Each year, with the changing climate, snowfall becomes more variable and the length or perhaps existence of the ski season more unpredictable. Being new to Spokane, I wanted to talk to local skiers and environmentalists to learn about the changes they've seen in snowpack, the length of the season over the years, and why they think skiing and winter recreation are important to Spokane.

One of the local skiers I interviewed is Heidi Lasher. Heidi and her family moved to Spokane about 13 years ago. She works as a consultant on communications and policy in Global Health and is also a writer with an MFA in Creative Nonfiction from Eastern Washington University. Heidi volunteered to work on the Spokane Climate Vulnerability and Resilience Assessment, a project which their website describes as "investigat[ing] how climate and weather-related impacts—including rising temperatures, wildfires, and the loss of snowpack—are projected to affect the community of Spokane, its businesses, working lands, and ecosystems in the decades ahead." Heidi's work on the project focused on the assessment of snowpack, “I worked on how snowpack would affect recreation, economy, and ecology. It was very interesting to just think through those scenarios and really think about how many businesses rely on winter recreation in this area and what is the link between snowpack and water.” Heidi says that when it comes to the future of skiing in Spokane, “unpredictability is the issue… we are so on the border of the rain-snow transition everywhere in this region, that it just feels like a roll of the dice.”

I also spoke with Professor of Recreation and Tourism Management, at Eastern Washington University, Matthew Chase. Matthew has been skiing for over forty years both for fun and at times as a career, as a professional ski patrolman (snow safety, evaluation, and evacuation) or a USAF Pararescueman. I asked Matthew about the changes he’s noticed skiing in this region, he said, “There are some winters where we get a lot of snow and then we get this period of rain and then we go back into snow. And then the classic one is it comes in as snow and then ends up as rain. You see that over and over again too. It just doesn't stay cold enough, long enough to produce.” For precipitation to fall as snow instead of rain, or for resorts to be able to make artificial snow, temperatures must be at or below 32°F. The Spokane Climate Vulnerability and Resilience Assessment found that “Mt. Spokane is projected to see 41 to 58 fewer freezing days by mid-century (2040–2069), compared to the last third of the twentieth century (1971–2000)” (Henning et al.). Fewer days below freezing means less likelihood of snow and a shorter ski season. But precipitation falling as rain instead of snow raises environmental concerns as well.

Greg Gordon, Professor and Chair of Environmental Studies and Sciences at Gonzaga University, and I spoke about how the difference between precipitation falling as rain and falling as snow affects the Spokane River and watershed. He explained, “If [precipitation] falls as snow, it stays put for longer…snowpack holds the moisture and releases it almost like a timed-release Tylenol… so that it lasts throughout the summer, rather than it being all off in the Columbia River and out in the ocean by the first of June. We're seeing more and more often that even if we get the full snowpack, it disappears so quickly compared to 20 or 30 years ago. The runoff occurs earlier and earlier every year.” A shortened ski season doesn’t only cause issues for people who ski or work on the mountain, as with everything relating to the environment, it is all connected. Higher winter temperatures mean less snowpack and earlier runoff, which affects the water cycle, impacting everything from lawn care to agriculture, to a more intense wildfire season, and so on. So even for those Spokane locals who wish the cold, dark winters away, and couldn’t care less about skiing or winter recreation, there are still plenty of reasons to join the skiers in their snow dances.

Greg grew up in Denver, Colorado, and spent all the time he could skiing at Loveland Ski Area where his father was a ski patroller. When he was looking at where to move after finishing graduate school at the University of Montana, Greg picked Spokane in part for the proximity to the mountains. He skis an average of 50 days a year. I asked Greg about what he’s noticed regarding snowpack over the last decade or so skiing in this region. He said, “What’s happening [in this area] is we're losing that low-elevation snowpack…We're losing everything from 5,000 [feet in elevation] down to 3,000 feet.” Temperature and elevation have a negative correlation, meaning that as elevation increases, the temperature drops, and vice versa. Lower elevations typically have higher temperatures, which means a higher risk of losing snow faster and seeing precipitation more likely to fall as rain than snow. Unfortunately, the five mountains surrounding Spokane top out with Schweitzer Mountain with a summit elevation of 6,400 ft (*OnTheSnow*). The lowest base elevation of the five ski areas is found at 49 Degrees North at 3,923 ft (*OnTheSnow*). The mountains and ski areas surrounding Spokane are operating between these two elevations, right in the danger range where temperature and snowfall are at risk in the upcoming decades. While other factors like latitude, longitude, and slope aspects come into play, elevation is a crucial factor. Greg said, “With the loss of that low-elevation snowpack. It's just shrinking the amount of available space at the same time the skiing demographic is growing and so you're seeing an increasing competition for a shrinking resource, which is sort of the state of the world for the 21st century, no matter what resource you're looking at.” If the surrounding ski areas lose too much snow, skiers in Spokane (who have the means) will need to start looking into traveling to mountains with higher elevations in order to ski, but how soon or when exactly this will happen could be anyone’s guess.

With skiing there have always been good seasons and bad seasons, weather patterns like El Niño and La Niña come into play, storms change direction, or end up dropping much more or much less snow than anticipated. Will the predicted rise in temperatures and change in precipitation make the bad snow years more frequent and the good years rare? It is looking that way, however just how soon we will start to see this happening on a year-to-year basis is unknown. In some ways, there has always been an unpredictability to the sport. Each person I spoke with remembered bad years, recalling the 2015 season specifically when several of the Spokane adjacent ski areas had to cut their seasons short due to lack of snow. But Heidi, Matthew, and Greg also cling to the seasons when the snow is good or even just fine, and they are up playing and exploring the snow-covered mountains. Matthew said "[for] the people who live in the Northern climates [skiing] provides access or opportunity for people still to get out and be active, enjoy the outdoors in the wintertime. And that's particularly the case for Spokane." Heidi echoed Matthew's sentiment, "[skiing] is a way to spend time out in nature in the darkest part of the year in probably the most beautiful setting because snow makes everything brighter and whiter. You're out using your body. You're not on your phone. You're not in a mall or a restaurant. You're just out having good, clean fun." They each also commented on the "lifetime" nature of skiing, highlighting that it's a sport that you can start learning as a three-year-old and continue into your nineties (if you stay in shape and in good health).

When asked about what Heidi hopes the future of skiing will look like, she said "My real hope is that my kids can continue skiing and their kids can. It doesn't have to be resort skiing. It's just like being out there and having fun and having a place to go in the winter and witnessing the beauty and craziness of snow. I hope they get to do that." I agree. I hope the next generations get to experience skiing and I hope we can find a way to make the sport more accessible and inclusive. The ski industry is changing, but it's a sport that has never lacked innovation. So, while we can theorize about what will happen over the decades to come, the only thing we know for sure is that the sport will continue to change and adapt, hopefully for the better. For now, we must consider the changes we can make on personal, business, and legislative levels to attempt to slow or change the current climate trajectory and keep skiing and ski culture alive. As we continue to develop solutions, we can look forward to the summer season, where these local ski areas are working to diversify their offerings with mountain biking, retail experiences, events, and beyond. And in no time at all, the skiers of Spokane will start again peering up at Mount Spokane each morning hoping to see it blanketed in fresh snow. I know that come the first big snow of the season, you'll find me standing out on the snowy sidewalk near downtown, skis on foot, thumb in the air, waiting for someone to round the corner and toss me a tow rope.

Aaron Hedge
Mentor: Rachel Toor

Federal Courts, a Native Tribe, and Reviving a Lake

The Coeur d'Alene Tribe of North Idaho works with Idaho's Department of Environmental Quality (DEQ) to manage the ecology of Coeur d'Alene Lake and other local waters. Together, they counter invasives, try to limit phosphorus in the lake, help landowners build better septic systems, and restore riverbanks. The state recently allocated $22 million in state and federal funds for such projects. Both governments say it's essential work. The lake feeds an aquifer from which 500,000 people drink. This cooperation reflects a national trend in which local, state, and federal governments increasingly draw on Indigenous knowledge to manage ecosystems. So why does the tribe want to sue Idaho for ownership of the lake?

Coeur d'Alene Lake sits within a massive environmental cleanup site, in which the feds are removing a century's accumulation of toxic metals pushed into streambeds from hard-rock mining in the Silver Valley. That work has been going for forty years, and it's not nearly done. The metals will never fully be removed from the lake itself, so they must be managed. The tribe, which used the lake for thousands of years before whites came to the region, thinks it can do the best job of any party. "The tribe is always gonna look after that lake," former tribal Chairman Ernie Stensgar said last year, "and always claim it."

The state and the tribe have different philosophies of water management. Native tribes often consider how any action they take now could affect future generations. White governments, by contrast, focus more on economic growth, which in North Idaho is strong. The Wall Street Journal in 2021 named the city of Coeur d'Alene the nation's "hottest housing market." Things seem good in North Idaho, but the tribe worries the state is blase about its ecological obligations.

This philosophical contrast is apparent in arguments between the tribe and state over the amount of phosphorus entering the lake. Phosphorus, which spikes with economic development, feeds algae that can deplete oxygen in water. In Coeur d'Alene Lake, too much phosphorus could cause a chain reaction that would free the toxic metals trapped in the lakebed and poison the water. In 2001, Gov. Dirk Kempthorne, promoting North Idaho tourism, drank a glass of raw lake water at a photo op and declared the lake pristine. But Stensgar, who also attended the event and had a glass, threw his drink back in. The tribe's limnologist said a year ago "there is too much phosphorus in the lake." Gov. Brad Little in 2020 commissioned a National Academies study on the lake's chemistry using data gathered by tribal and state scientists. After the study was published last year, saying the lake was for now safe, the tribe seemed hesitant to celebrate the news, focusing instead on the report's calls for further investigation of the lake's chemistry. The state and the tribe must "increase our project work around the lake as well as to follow up on the studies suggested by the report," said the tribe's head of natural resources, Caj Matheson, in a DEQ press release. "We are seeing some positive trends but those trends are short and the report also shows that it is the lake's oxygen levels that, if they are too low, will trigger the release of those metals from their rest on the bottom of the Lake into the main water column."

Before first contact the tribe used the lake as a canoe highway, moving venison and berries in summers to prepare for harsh winters. After a 2001 Supreme Court ruling, the tribe owns the lake's southern third, but Idaho controlled the lake before that year. Water highways work well for mining, and in the 1880s, a silver rush occurred in the region during negotiations between the United States and the tribe to establish the Coeur d'Alene Reservation. The first serious iteration of the reservation encompassed the whole lake, excluding a tiny portion of the north end. The miners, needing the lake's waterways to transport equipment to extract the discovered riches, didn't want to deal with the tribe when it moved inventory. So, Congress moved the reservation's northern border just south of the Coeur d'Alene River's mouth so miners could enter it. The United States paid $2.50 an acre for this land, but "they never compensated the tribe even a nickel for any of the waterways," said Howard Funke, a Coeur d'Alene attorney who has represented the tribe in water-related lawsuits, in a documentary interview.

As land purchases so often do, this one sparked a shift in the environment. After millennia during which the tribe safely moved food over the water in canoes, literal mountains of toxic heavy metals began washing down the Coeur d'Alene River and into the lake from the Silver Valley, where hills were most radically mined. In the 1930s, cattle farmers noticed their livestock becoming sick from the water. Tribal leader Henry SiJohn told High Country News in 1996 that tribe members "would hear the death cries of wild animals and find the carcasses and they concluded something was wrong with the water. We didn't have any data or statistics to go on. All we had were the five senses." In the 1970s, researchers found the highest concentrations of lead in human blood ever recorded in Silver Valley children. The rivers were essentially dead.

In 1909, the feds divided the reservation into 160-acre parcels, giving one to each tribal member in an "allotment." (Allotment devastated tribes because it hollowed out tribal autonomy.) Much reservation land was left over, and the government auctioned it off, leaving the tribe 345,000 acres of a once-four-million-acre territory. "The tribe kind of went dark," Funke said. But as mining pollution, tucked in the mountain valleys, appeared in the lake itself, the tribe felt revitalized by the urgency of this threat. In 1990 it asked mining companies to clean up the water and Idaho to give it back the lake. But the talks failed, so the tribe sued the miners for damages. It also sued Idaho to claim the lake for itself in a suit referred to as "*Idaho I*"; if it owned the lake, it could get more cash in the suit against the mines. Maybe it could win enough to clean the metals from the lake under new federal environmental provisions.

“It’s a hundred-plus-year-old case for us,” said Paulette Jordan, a Coeur d’Alene Tribe citizen and former member state legislator, in a documentary. When miners “started extracting the resources and then dumping their tailings into our water systems … the tribal peoples decided to take action because neither the state nor the federal government were stepping in.”

Idaho, I became a remarkable case study in states’ legal strategies and protections against tribes. The Eleventh Amendment of the Constitution says states are sovereign governments, immune to suit in federal court from other sovereigns. The Coeur d’Alene Tribe is such a sovereign. A federal court ruled the tribe could not sue Idaho in federal court because of the state’s constitutional immunity, and the case went to the Supreme Court. The tribe could have sued in state court, but the state would have ruled in its own favor, Funke said. So, the status quo that the state owns the lake would endure for another decade.

As *Idaho I* navigated federal courts, it became clear the tribe would not succeed, so Funke asked the United States Department of Justice to file its own lawsuit on the tribe’s behalf. Idaho is not immune from the feds in United States court, but there was a hitch. “They said in 1899, 'We bought this from you,’” Funke said in the documentary, indicating the northern parts of the original reservation on a map. “‘We’re not going to claim that as part of this lawsuit against the state of Idaho; we’re only going to claim what’s in [the reservation] boundary.” Funke said a suit by the feds saying the tribe owned the entire lake would have amounted to admitting the states had stolen the lake from the tribe, so the smaller scope became the only way forward.

In 1997, the Supreme Court refused to hear *Idaho I* because of Idaho’s Eleventh Amendment immunity. But Idaho faced DOJ’s lawsuit, “*Idaho II*,” and federal lawyers, scholars of Indigenous law, and the tribe crafted a case that swayed the high court to declare the southern third of the lake the tribe’s. This decision came after the mining companies were deemed liable for the polluted basin. These were huge victories for tribes. The EPA used the more than $600 million settlement with the mines to begin what’s now the second-largest environmental remediation in the United States. Much of the Coeur d’Alene waters is now crystal clear. Trees grow anew. People fish and gather water potatoes, and in summer tourists dot the lake’s beaches.

But in parts of the Silver Valley, streams still snake through mine tailings much taller than a person. There the pollution’s scope is evident: millions of tons of soil hot with heavy metals. The settlement is spent, leaving billions of dollars of work up to the EPA. And the agency won’t address the toxic sediments at the bottom of Coeur d’Alene Lake because Idaho convinced it to exclude the lake when it designed the cleanup, although the lake sits squarely in the site.

The question of who owns the lake’s northern two-thirds hasn’t been litigated, and the tribe remains unhappy with white governments’ resource management practices. But it won’t stop fighting to win back the lake. It’s reacquired much of its old land, including waterfront where children play in the lake. “We spent over $10 million in land acquisition, which is a pretty good chunk of property,” Stensgar said in 2022. “The United States better recognize it because we’re going to come back,” he said later. “We’re gonna sue again for the whole lake.”

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Thurman Johnson
Mentor: Rebecca Brown

The Restoration of Species on the Palouse Prairie

With over 99.9% of the Palouse prairie lost to land conversion, restoring native plant communities is crucial for ecological function, however, research on Palouse prairie restoration methods is sparse. Seed-based restoration uses a mix of seeded species to enhance competition against weeds, diversify vegetation, and adapt to environmental conditions. However, many factors can be varied, such as seed mix diversity and composition, the proportion of forbs to grasses, and seeding rate, and the most effective levels of each are not clear. Further, soil amendments, such as biochar, may benefit properties of tilled soils, but have not explored in Palouse Prairie systems. To address these knowledge gaps and inform Palouse Prairie restoration efforts, I conducted an investigation composed of 2 experiments. For experiment 1, my objectives were to test the hypotheses that (1) increasing levels of seed mix diversity, forb content, and biochar treatment amount would each increase native plant cover and species richness and decrease non-native plant cover and richness in the first-year after establishment, (2) biochar and topography would interact to increase native cover in dry areas with higher biochar treatment, and (3) moist topographic zones would improve restoration outcomes. For experiment 2, my objectives were to test the hypotheses that (1) higher seeding rate would increase native cover and reduce non-native cover and richness, and (2) species identity would interact with topography and seeding rate to affect native and non-native establishment.

My experiments were established on a 15-acre pilot site of Eastern Washington University's Prairie Restoration Project in Cheney, Washington. For experiment 1, 4 seed mixes were drill-seeded in a randomized block design across a topographic gradient. Biochar treatments of low (600 lbs/acre) and high (6000 lbs/acre) were overlaid onto the seed mixes. The four seed mixes included 1) 6 grasses, 2) 6 grasses with low diversity (8 spp.) forbs at 70% grass and 30% forbs, 3) 6 grasses with low diversity (8 spp.) forbs at 60% grass and 40% forbs, and 4) 6 grasses with high diversity (15 spp.) forbs at 70% grass and 30% forbs. For Experiment 2, I broadcast-seeded 4 common Palouse Prairie restoration species in monoculture, 1 equal parts blend of the species, and an unseeded control across 4 seeding rates: 861, 1615, 2368, and 3122 pure live seed per m^2. Sampling for both experiments took place in late June 2022.

In Experiment 1, native species richness and cover, and non-native cover were only affected by topography, but not other factors. Seed mix diversity and topography interacted to affect non-native species richness, which was lower in swales than north slopes across treatments but higher in north slopes than top/south in grass-only treatments. Topography was a dominant influence on first-year outcomes, which were not significantly affected by grass-forb composition. While biochar did not affect native or non-native species richness or cover, it did affect community composition. As biochar treatment level increased, the difference in community composition between topographic groups also increased. In Experiment 2, higher seeding rates did not clearly improve restoration performance. Instead, species identity and topography played key roles. Treatments of *C. pulchella* and *A. millefolium* led to more native cover than *G. aristata* or *L. lewisii*. Surprisingly, the blend treatment did not result in more native richness than the single-species treatments. For both experiments, future sampling is needed to test the longer-term effects of these mixes. My results indicate that (1) topography should be used to inform seed blend composition, (2) approaches outside of seed mix strategy may be necessary for suppressing invasive species, (3) biochar interacts with dry topographic zones to influence species composition, and (4) high-rate plantings may not be necessary for every species.

Together, my two experiments contribute to a deeper understanding of the complex dynamics involved in ecological restoration and seed mix strategies for the Palouse Prairie. By examining the interacting effects of various factors such as seed mix diversity, composition, biochar addition, seeding rate, species identity, and topography, these experiments provide valuable insights for restoration practitioners and decision-makers.

My first experiment highlights the significance of considering topographic variation when designing seed mixes. The identification of the dry hilltop/south slope topographic zone as conducive to better native performance emphasizes the importance of site-specific considerations in restoration planning. This knowledge may help practitioners to tailor their restoration efforts based on the specific characteristics of each zone, ultimately increasing the chances of successful establishment and persistence of native species. Furthermore, the interaction between biochar and topography revealed in my experiment offers a potentially promising avenue for modifying species composition in dry sites. The ability of biochar to influence the association of certain species within specific topographic zones, if understood more thoroughly, could provide an innovative approach to favoring desirable species and managing invasive species. Further research and experimentation could explore the optimal application of biochar in specific topographic zones, fine-tuning its potential as a tool for ecological restoration.

In my second experiment, the significance of species selection in seed mix design was made evident. The superior performance of *Clarkia pulchella* and *Achillea millefolium* underscores the importance of choosing species that are well-suited to the restoration site's conditions. My findings suggest that incorporating a careful selection of species with proven adaptability and compatibility with the target site is a valuable consideration for achieving successful restoration outcomes.

Moreover, my second experiment highlights the need for continued research to evaluate the performance of additional species. By expanding the pool of species tested, researchers can gain a more comprehensive understanding of the potential species combinations that can maximize native cover and richness in the Palouse Prairie. This knowledge will enable restoration practitioners to make more informed decisions when designing seed mixes, improving the effectiveness and efficiency of restoration efforts.

In summary, my two experiments together provide valuable insights into the intricate relationships between seed mix design, topography, and species selection for ecological restoration in the Palouse Prairie. My findings emphasize the importance of site-specific considerations, the potential benefits of biochar as a restoration tool, and the significance of selecting species that are well-adapted to the site conditions in which they are planted. By integrating these insights into restoration practices, we can enhance the success of restoration efforts and promote the recovery and preservation of the unique ecological communities found in the Palouse Prairie.

Devin Mumey
Mentors: Giovanna Bishop MS, Jessica L. Allen, PhD

Lichens of Iller Creek

INTRODUCTION

The Iller Creek Unit of the Dishman Hills Conservation Area, initially called the Dishman Hills Natural Area, was acquired by Spokane County and the Dishman Hills Natural Area Association in several parts starting in 1994, the first being a $225,000 purchase of 170 acres of land from private seller Barbara Olsen, which encompasses the Rocks of Sharon and surrounding area. In the same year, the Natural Area expanded via a Washington State Grant of $500,000. From 1995 to 2011, purchases by the County and DHNAA (now called the Dishman Hills Conservancy, DHC) in addition to generous donations of land from various local families increases the total land allocated to the Dishman Hills Natural Areas to 1,159 acres, of which 966.2 acres are currently designated as the Iller Creek Unit.

In the August 1995 Management Plan published by the Washington State Department of Natural Resources, the purpose of a conservation area is to maintain the scenic landscapes and habitats of sensitive, threatened, or endangered species, as well as providing an educational area for low-impact public use. Currently, the DHC-approved activities include birdwatching, wildlife viewing, hiking, biking, cross-country skiing, and snowshoeing. Heavy-impact activities such as horse-riding and snowmobiling are currently discouraged. The 1995 plan also contains lists of various animals, plants, and fungi found in the ecosystem in the Iller Creek Unit and describes an ambiguous goal of future researchers completing more extensive checklists on the total biodiversity of the area. As of 2023, there have been no full-scale studies completed in the Iller Creek Unit.

The Iller Creek Unit is a unique area, with a diverse range of habitats. There are various riparian environments, with dense and shrubby underbrush, understories of deciduous trees, and an abundance of Ponderosa pine trees. Surrounding these lush green areas are dry savannahs, populated by tall grasses and drought resistant bushes. There are also large xeric rock outcroppings, such as the popular climbing area Rocks of Sharon, from which many of our samples have been collected. This area, which is sandwiched between the growing cities of Spokane and Spokane Valley, gives a unique opportunity to study not only the diversity of organisms in various habitats, but also to track the effects of human expansion and recreational use on the diversity of many biomes, while keeping the study area small-scale and easily observable.

The goal of this project was to perform a preliminary study of the lichen population in a small portion of the Iller Creek Trailhead, to assess the feasibility and resource requirements of a full-scale lichen checklist of the entire Iller Creek Unit. Creating a comprehensive checklist and collecting representative samples of all species present will not only give vital information on biodiversity for the continued conservation of the area, but also create a collection of physical specimens that can be used to create a baseline for said biodiversity and air pollution levels, which can further be used in comparison to metrics in future studies of the area to extrapolate changes in the environmental conditions.

METHODS

Standard identification techniques were used for this project, including the use of dichotomous keys written by experts in the field, spot tests using various chemical reactants, and thin-layer chromatography.

Dichotomous keys contain couplets, each describing a morphological feature or chemical reaction that distinguishes one species of lichen from another. By choosing the corresponding feature, the couplets lead through a path of identification differentiating between similar species until you arrive at a point where the singular species can be confidently determined. While a large number of these couplets compare features such as growth form (i.e., crustose, leprose, foliose, fruticose, dimorphic, etc.), color, size, reproductive mode and structures observed, and spore morphology, they also compare various chemical reactions (spot tests) which help to determine the various secondary metabolites produced, many of which are completely unique to lichens.

Spot tests are a common way to determine the secondary metabolites produced by lichens. Using various reagents, such as chlorine, potassium hydroxide, and an ethanol-p-Phenylenediamine solution are applied to various parts of the lichen and observed for a color change. A positive reaction is indicated by the natural color of the lichen structure changing, whether to yellow, orange, red, or purple. Each color corresponds to the presence of a chemical. Using the color change, we can follow the dichotomous key couplets to a definitive species identification.

Further identification of secondary metabolites can be done using thin layer chromatography (TLC). A piece of the lichen is mixed with a 95% ethanol solution, which extracts the secondary metabolites and allows us to micropipette the chemical product onto a thin aluminum sheet coated with silica. Various solvents are used for TLC. For this project, solvent C was used, which is a mixture of 18% toluene and 82% glacial acetic acid. Approximately 100 ml of the solvent is poured into a large glass beaker, and then silica-coated plate with the extracted metabolites is placed vertically into the solvent. Due to the polarity of the solvent and the metabolites, as the solvent is absorbed by the silica and moves up the plate, the various metabolites are carried with it and deposited in specific spots on the plate. After removing the plate from the solvent and drying, it is processed in several ways. The first is observing the color of the spots under natural light, then under long and short-wave UV light. The plate is then wetted and dried on a warm surface to allow observation of any hydrophobic spots. Once fully dried, a 10% hydrochloric acid solution is applied, and the plate is heated for approximately 3 hours (a process called acid burning) which brings out additional coloring. The plate can then be observed again under long-wave UV light. Each of these steps is photographed, and then compared to standards found in various books and research papers written by field experts.

The species identified thus far include:

Alectoria sarmentosa
Aspicilia cf. contorta
Bryoria fremontii
Calicium viride
Caloplaca arenaria
Candelaria pacifica
Candelariella rosulans
Candelariella vitellina
Carbonea vorticose
Chaenotheca furfuracea
Chaenothecopsis subparoica
Chrysothrix chlorina
Cladonia fimbriata
Cladonia ochrochlora
Diploschistes muscorum
Diploschistes scruposus
Esslingeriana idahoensis
Evernia prunastri
Fuscopannaria cyanolepra
Hypogymnia austerodes
Hypogymnia imshaugii
Hypogymnia physodes
Hypogymnia tubulosa
Lecanora carpinea
Lecanora cenisia
Lecanora confuse
Lecanora pacifica
Lecanora polytropa
Lecanora rupicola
Lecidea cf. atrobrunnea
Lecidella stigmatea
Lepraria neglecta
Lepraria rigidula
Lepraria torii
Leprocaulon knudsenii
Letharia Columbiana
Letharia vulpine
Lichenoconium cf.
Massalongia carnosa
Melanohalea subelegantula
Montanelia disjuncta
Mycoblastus sanguinarius
Parmelia hygrophila
Parmelia saxatilis
Parmelia sulcata
Parmeliopsis ambigua
Peltigera Britannica
Peltigera canina
Peltigera malacea
Peltigera praetextata
Phlyctis argena
Physcia aipolia
Physcia caesia
Physcia dubia
Physcia magnusonii
Physcia phaea
Physcia tenella
Physconia enteroxantha
Platismatia glauca
Platismatia wheeleri
Pleopsidium flavum
Polycauliona polycarpa
Protoparmeliopsis garovaglii
Protoparmeliopsis muralis
Psora nipponica
Psuedephebe pubescens
Ramalina farinacea
Rhizocarpon bolanderi
Rhizocarpon disporum
Rhizocarpon grande
Rhizocarpon macrosporum
Rhizoplaca melanophthalma
Rinodina confragosa
Rinodina confragosa
Schaereria fuscocinerea
Tuckermanopsis chlorophylla
Tuckermanopsis platyphyla
Umbilicaria americana
Umbilicaria hyperborea
Umbilicaria phaea
Umbilicaria polyphylla
Umbilicaria polyrrhiza
Umbilicaria vellea
Usnea lapponica
Vulpicida canadensis
Xanthomendoza montana
Xanthoparmelia coloradoensis
Xanthoria Candelaria

By combining the information gathered from various lichen identification techniques, a positive identification can be made for nearly all lichens. However, to be certain of the identification, DNA extraction and barcoding is done. As this project is ongoing, next steps will include DNA barcoding of all samples collected and identified to date, as well as continued collection in the areas left unsearched. With nearly 1000 acres to search, and the recent announcement of the potential purchase and addition of 100 additional acres, this project is certain to be expanded and continue for several years more.

Claire Richardson
Mentor: Dr. Carmen Nezat

Elemental Concentrations in Urban and Rural Dust in Spokane, WA

Introduction

Particulate matter in the air we breathe is known to have adverse health risks and the Spokane area has experienced patterns showing high PM_{10} concentration levels that reflect annual and seasonal trends (Air Pollutants and Trends, 2023). Particulate matter in our atmosphere measuring less than 10 micrometers is known as PM_{10}, and includes dust, pollen, and mold which are released as tiny droplets of liquids or solids by certain sources including dust from construction and agriculture, vehicles, and outdoor burning (Department of Ecology, 2022).

In addition to the total amount of PM_{10} in the atmosphere, its toxicity could be linked to its elemental concentrations. Studies that explore the elemental concentrations of PM_{10} have been conducted in countries other than the United States that are more likely to experience low air quality and adverse health effects (Huiming, Li et al, 2013) but are limited in the US. Exposure to heavy metals in particulate matter can have adverse health effects including cardiovascular and respiratory diseases (Jianghanyang, Li et al, 2021). The major and trace elemental concentrations of PM_{10} include Na, K, Mg, Ca, Fe, Al, Mn, and Mo which are likely from

mineral dust and Pb, Sr, Cu, Ba, Ni, As, and V which are from anthropogenic sources (Li, Jianghanyang et al, 2021). The objective of this study is to compare the heavy metal content of

PM_{10} between urban and rural locations (city of Spokane and Turnbull National Wildlife Refuge, respectively) over the same time period.

Methodology

The Spokane Regional Clean Air Agency (SRCAA) maintains seven air quality monitoring stations that measure total levels of particulate matter measuring less than 10 micrometers in the Spokane County, including locations in the city of Spokane and Turnbull National Wildlife Refuge. This research analyzed the elemental concentration of PM_{10} from air filters from the Spokane Regional Health District located at 1101 W College Ave, Spokane, WA and Turnbull National Wildlife Refuge at 26010 S Smith Rd, Cheney, WA. This particulate matter was collected over a 24-hour period using Tisch General Metal Works High Volume PM_{10} Sampler (Cruz, 2022), and samples from multiple locations over multiple years have been provided to the EWU Geochemistry Lab by SRCAA. PM_{10} filters were chosen from three days per month over a period of five months (May, June, July, September, and October) in the years 2003 and 2004 from both Turnbull Wildlife Refuge (which represented the rural data) and the city of Spokane (for urban data). Both locations were sampled on the same day which allowed for chemical comparison of rural and urban particulate matter. I prepared sixty samples for analyses using the US EPA Method 3051 which entails digesting a sample in concentrated HNO_3 using a microwave digestion system (CEM MARS Microwave Digester) and filtering the digests through a 0.45 μm membrane. These digests were analyzed using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) to determine the concentration of multiple elements including major ions (Ca, K, Mg, Na) and heavy metals (Cu, Pb, and Zn; US EPA Method 200.7).

The objective of this study was to compare the urban data to the rural data to determine any patterns or correlations. I expected to see higher heavy metal concentrations in urban areas like Spokane compared to rural land in the Turnbull Wildlife Refuge because those samples were collected in areas with more point source and non-point source pollution compared to a less populated area.

Results

For all sixty samples analyzed by the ICP-OES, the atmospheric concentration of the particulate matter was measured for major ions (Ca, K, Mg, Na) and heavy metals (Cu, Pb, and Zn) and converted from mg/L to ng/m^3. The highest, lowest, and average values for each element were calculated for both locations. The urban data were compared to the rural data to determine any patterns or correlations. Heavy metal (Cu, Pb, Zn) concentrations in atmospheric dust were higher in urban areas like Spokane compared to rural areas like the Turnbull National Wildlife Refuge (Figure 1.a) due to the greater frequency of point and non-point sources pollution in more populated areas. In Figure 2, the concentrations of these elements overlap for the two locations suggesting a similar source of particulate matter for calcium and sodium, both common elements found in dust generated from local rocks and soils. Figure 3 shows that the Spokane Regional Health District has higher Zn concentrations for the same amount of Ca at the Turnbull National Wildlife Refuge. Common sources of zinc in urban environments include road dust consisting of tire wear, road surface wear, break wear and clutch wear (Adamiec, 2016). Figure 1.a shows three common elements generated by man-made activities are found in higher concentrations at the urban location (Spokane Regional Health District). Elements in Figure 1.b are common in rocks and soils including those found locally and thus can be used to represent the natural composition of local dust found at both the urban and rural locations. This data shows that calcium concentrations are higher at the urban location suggesting a possible man-made source like concrete may be a contributor to these elemental concentrations in the Spokane area. Local lead concentrations (Figure 1.a) are well below the US standard set of 150 ng/m^3 for lead concentrations in ambient air (Lead (Pb) Air Pollution) and zinc, iron, copper, and lead concentrations for both locations were much lower than the ambient air quality standards set by the Ontario Ministry of the Environment, Conservation, and Parks (2021) (Table 1).

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Tables and Figures

Figure 1: Concentrations of metals in the atmosphere (units in nanograms of element per cubic meters of air). (a) Cu, Pb and Zn and (b) Ca, Na, Fe, K, and Mg at both locations.

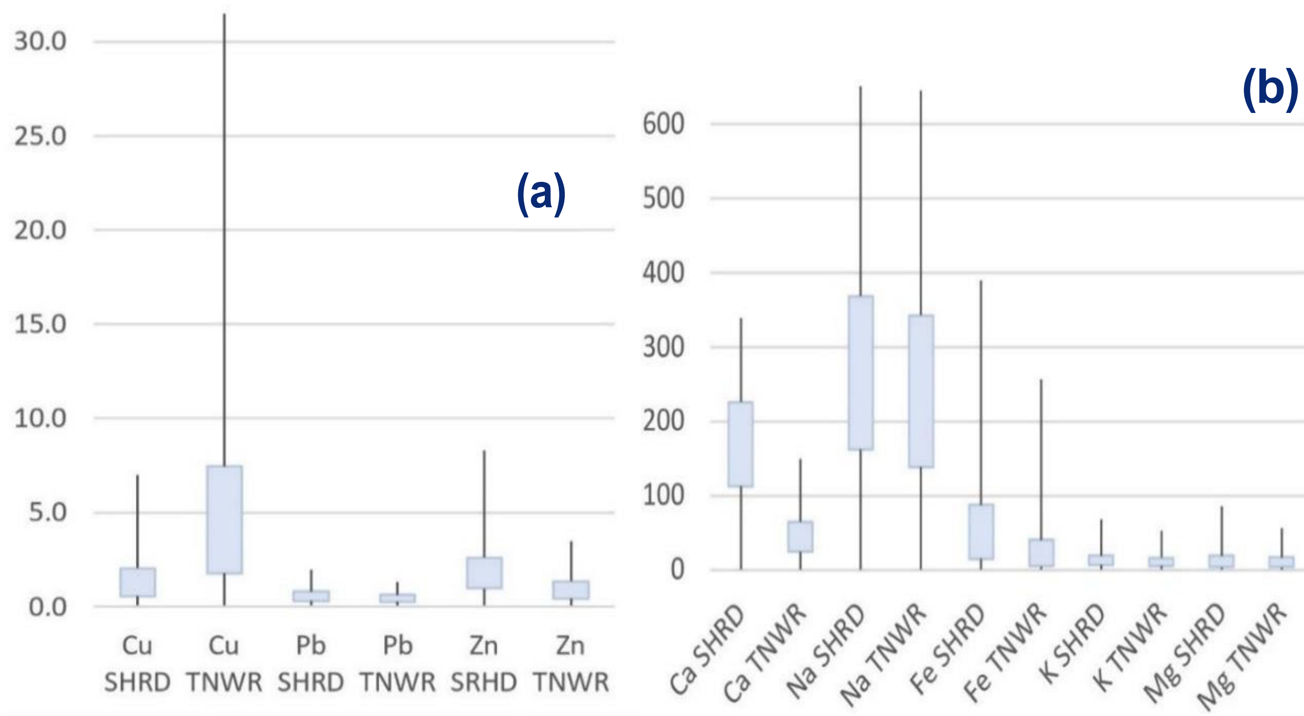

Figure 2: Concentration of Ca versus Na in particulate matter at both locations.

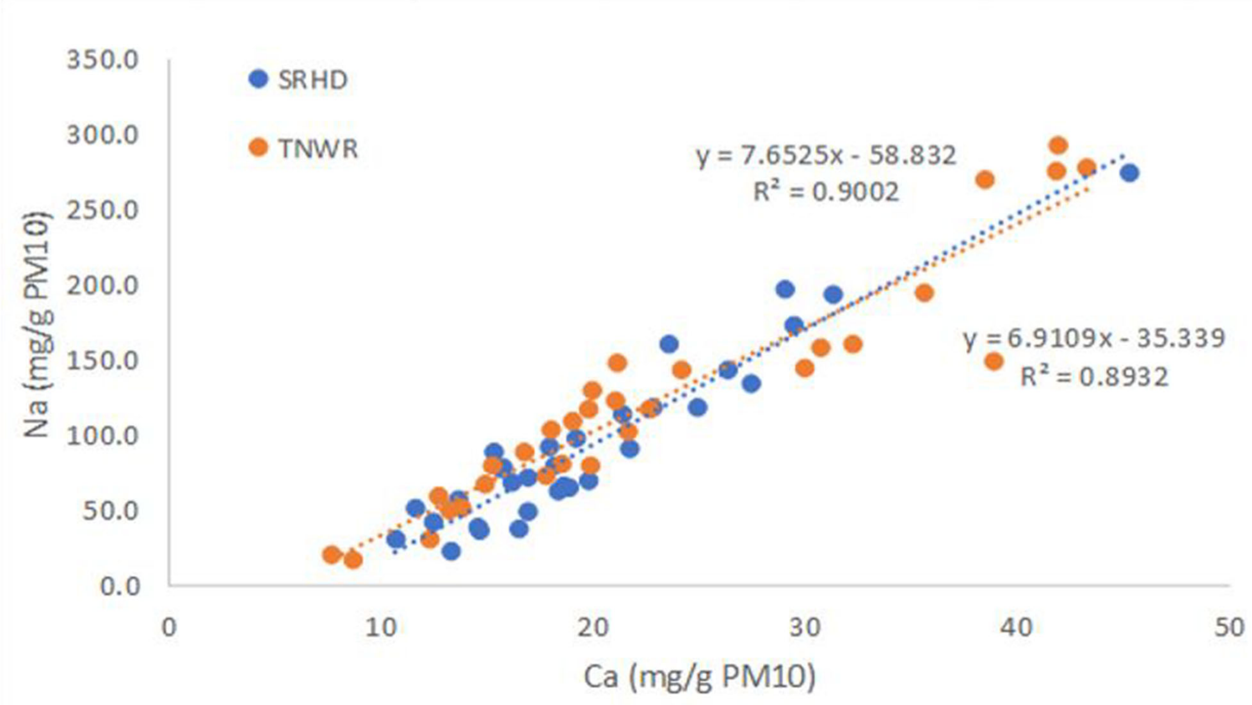

Figure 3: Concentration of Zn versus Ca in particulate matter at both locations.

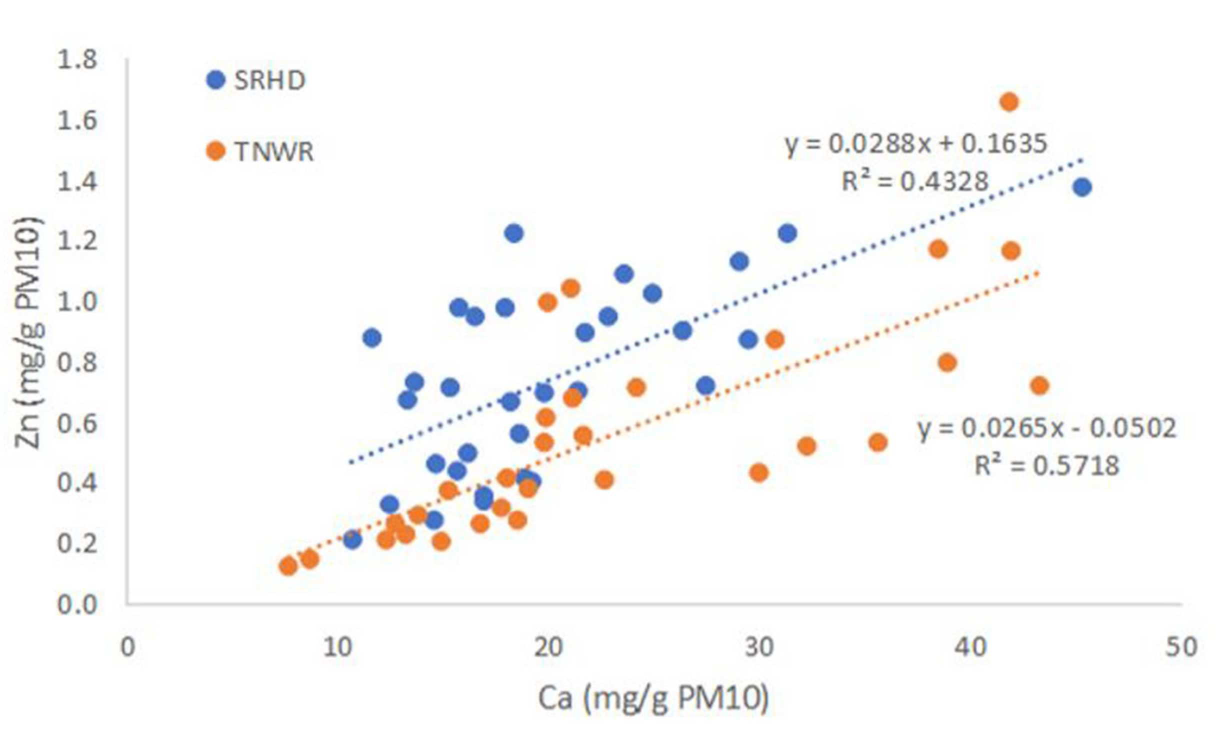

Table 1. The Ontario Ministry of the Environment, Conservation, and Parks (2021) and the concentration of select elements in the Spokane location and Turnbull National Wildlife Refuge.

Element	Ambient Air Quality Standard (ng/m^3)	Atmospheric Concentration Ranges in SRHD (ng/m^3)	Atmospheric Concentration Ranges in TNWR (ng/m^3)
Zinc	120000	0.9897-5.6901	0.4434-1.9645
Iron	4000	14.9666-302.4666	4.5591-217.1188
Copper	50000	0.5624-4.9349	1.7832-22.5244
Lead	500	0.3642-1.1571	0.2447-0.6518

Erin Toulou
Mentor: Dr. Chad Pritchard

Modeling Groundwater Toxin Flow in the West Plains

This study focuses on modeling where groundwater flows in West Plains, eastern Washington to understand the distribution of contamination. The city of Airway Heights resides in this area and is a large focus on this project. Dr. Chad Pritchard was approached by a local resident from this area concerned about their health and safety, as well as their neighbors. I began helping Dr Pritchard with this research during Spring quarter 2022. Our plan is to model the West Plains to get a better understanding of how the groundwater distributes PFAS, or per-or poly-fluoroalkyl substances, which is a manufactured substance that is a wonderful water-replant, so it is found in Gor Tex, Kevlar, paints and waxes, photographic materials, packaging and a host of other materials. However, the most common source of PFAS in the news seems to be associated with AFFF fire retardant that can put out gasoline fires, and was heavily used at airports. In 2017, PFAS was detected in drinking water in Airway Heights and surrounding residents, due to flame retardant (AFFF) used at Fairchild Airforce Base. PFAS has been in the headlines and news articles locally worldwide because it is a toxin that can cause liver damage, cancer, and a host of other human and environmental concerns.

Understanding groundwater movement in the West Plains could potentially help with contaminant transport and could identify local hotspots other than Fairchild Airforce base. I helped Dr Chad Pritchard filter out and enter well log data from the Department of Ecology well database. After grouping this data, we uploaded it into ArcGIS Pro and created models of a map with all the wells we filtered out, a map of wells with common upper restrictive layers, and created a Darcy flow model. Also, using former students' data, we included pie charts of areas that were tested positive for PFAS with the compositions of each locality.

I presented this research at EWU Creative Works Symposium 2022, Murdock College Science Research Conference 2022 in Vancouver Washington, I spoke at an EWU Geosciences Seminar in 2022, the Northwest Groundwater conference 2023 in Pasco Washington, Medical Lake City Council 2023, GSA 2023 Cordilleran Section meeting in Reno Nevada, and EWU Research and Creative Works Symposium in 2023.

I appreciate being recognized for this award and I cannot wait to see how this grant will help future students' research! Thank you for this opportunity.

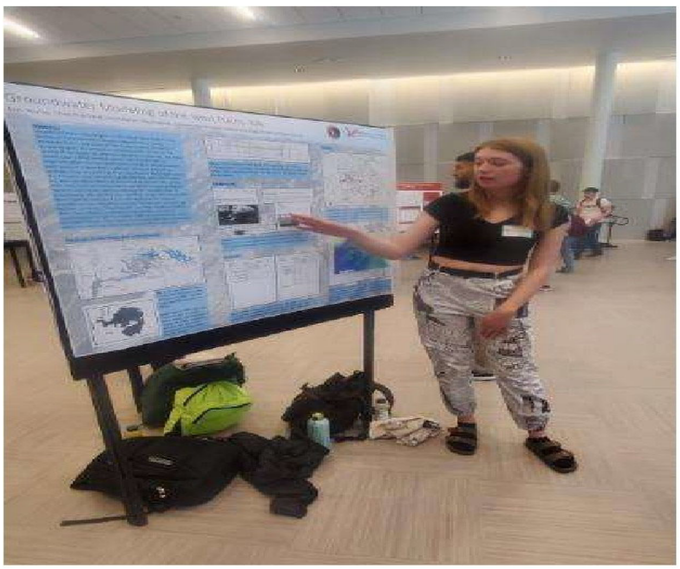

Figure 1: EWU Research and Creative Works Symposium 2022

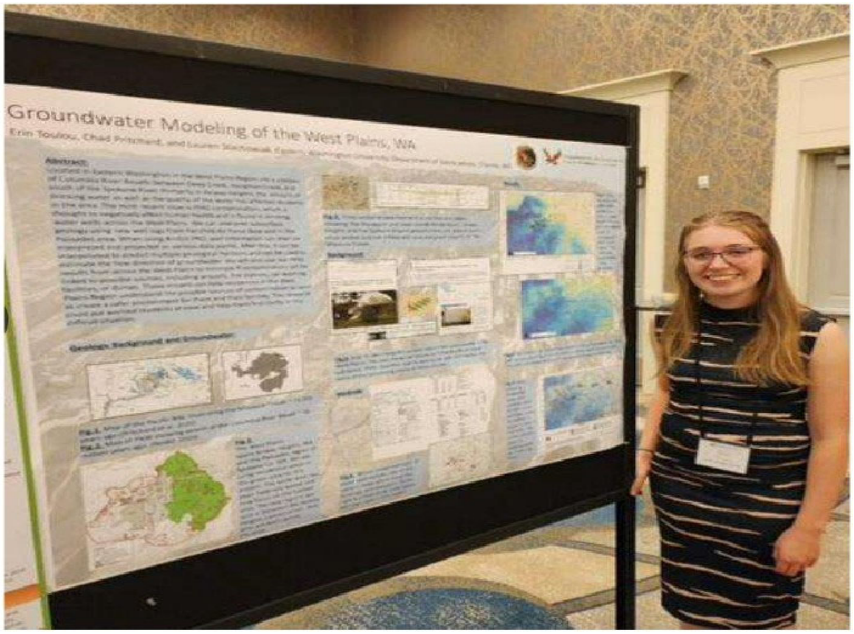

Figure 2: Murdock College Science Research Conference 2022 in Vancouver, WA

Figure 3: Northwest Groundwater Conference 2023 in Pasco, Washington

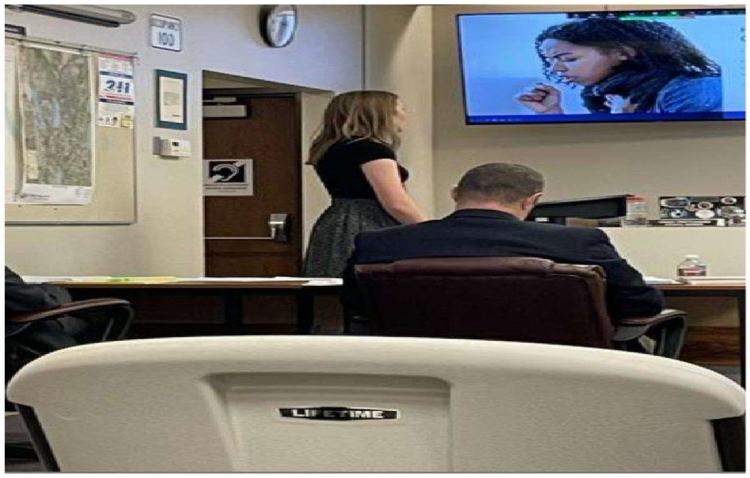

Figure 4: Medical Lake City Council 2023

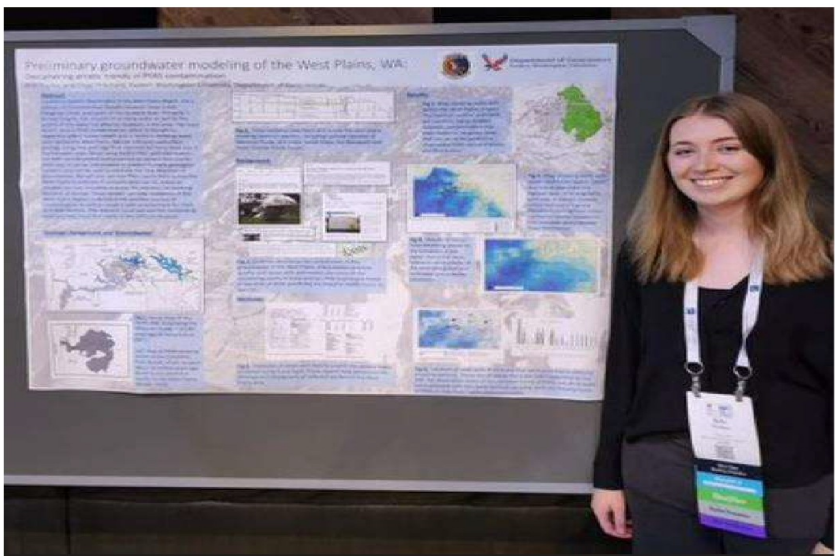

Figure 5: GSA 2023 Cordil/eran Section meeting in Reno, Nevada