Proposal Description

PCBs Toxicity

PCBs or polychlorinated biphenyls are man-made chemicals that are persistent and ubiquitous in nature (Montez & Peterson, 2017). PCBs are the last to be degraded in the environment, and they are harmful to the food chain, which negatively impacts humans ("PCBs in Municipal Products REVISED", 2015). Before 1977, many manufactured products contained PCBs such as light fixtures, transformers, and road paint. Although the manufacture of PCBs has since been prohibited in the U.S., PCBs persist in the environment, and can be produced inadvertently, posing a large health risk to those who interact with the chemicals ("Polychlorinated Biphenyls (PCBs) - Public Health", 2013). Some of these health risks are immune deficiencies, birth defects, reproductive failure, and cancer. ("Polychlorinated Biphenyls", n.d.).

Bioremediation using fungi vs Native consortium

Bioremediation is the process of using microorganisms to remove waste from the environment and can be subclassified by the method of remediation. Biodegradation is the enzymatic recycling of toxic molecules into less harmful ones. Biosorption is the binding and removal of metallic pollutants, and bioconversion is the conversion of waste into a usable form such as mushrooms (Kulshreshtha et al., 2014).

Studies have shown that specific species of Bacteria and Fungi can isolated and used to remediate toxic compound in the environment, and several have been employed to do just this. Specifically, genera of white rot fungi such as *Phanerochaete*, *Trametes*, *Bjerkandera*, and *Pleurotus* have been used to remediate harmful compounds such as textile dyes, PAHs, chlorophenols, TNT, pesticides, and nylon (Korcan, Cigerci and Konuk, 2013). Likewise, dozens of species of bacteria have been identified to remediate heavy metals, dyes pesticides and hydrocarobons (Gizaw and Wassie, 2017). In particular, the genus *Rhodococcus* is well-known for its ability to biodegrade PCB and hydrocarbon compounds. Frederick et al. (2020) used Oxford Nanopore Technologies and Illumina technology sequencers to sequence the complete genome of the nitrile biocatalyst *Rhodococcus rhodochrou*s ATCC BAA-870. It was estimated that "As much as 74% of genes may have unknown metabolic functions" (Frederick et al., 2020, p. 14), indicating significant bioremediation potential.

While individual species are clearly important for the bioremediation process, many researchers have found that their individual capability is often dependent on a number of factors, not the least of which is the presence of a symbiotic microbial consortium (Lee et al., 2018). In fact, several studies have shown that the native consortium of soil microbes often evolves the capability to degrade toxins without bioaugmentation of "super fungi" (Silva et al., 2009)

History of Project at NC

Applying the theory that the native consortium has evolved the capability to degrade PAH's, beginning in 2018, North Central High School began conducting several pan studies in collaboration with Les Stephens and Mike Petersen. These studies from the North Central High School Institute of Science and Technology (IST) lab have consistently shown significant reductions of PCBs to undetectable levels in contaminated soil. (Unpublished report, Harwood & Heimbigner, 2019) (Unpublished report Thang & Toney, 2020)

Most recently, our lab sought to characterize the changes in soil consortium using Next generation sequencing. A small-scale pan study was carried out using PCB contaminated sediment collected by the city of Spokane and placed in 3 pans. Pan A contained sediment with no amendments, Pan B contained sediment with nitrogen fertilizer at a 400:1 C/N ratio, and Pan C contained sediment with nitrogen fertilizer and an inoculant of remediated sediment from a previous experiment. Pans were aerated daily for three months, and nitrogen fertilizer was added again halfway through the experiment. Samples were taken at the beginning of the experiment and monthly thereafter for analysis. PCB levels were determined using Method 8082A by Gas Chromatography and TPH was determined using NWTPH-Dx. The bacterial consortium of each sample was profiled using Oxford Nanopore Next Generation Sequencing. After three months, Pan B saw an 81% decrease from initial PCB levels while Pan C saw a 63% decrease. Over three months, some genera of bioremediating bacteria such as *Rhodanobacter, Clostridium,* and *Nitrospira* increased while PCB levels decreased. Our results demonstrate that bioremediation can be achieved, at least at the small scale, by encouraging the growth of native soil bacteria using nitrogen fertilizer and oxygen.

Benefit to Finding and Reducing PCBs

PCBs in Spokane Vactor Waste

The Spokane Vactor Waste facility is located at the northwest corner of Playfair Industrial Park. The vactor waste is removed (vacuumed) from stormwater drains throughout the city and taken to the site. The fenced in site has a covered but open sided structure, where the vactor waste is dumped. Of the waste that is brought to the site, about 900 tons/year are hauled to the landfill. An average of 1500 tons of liquid are decanted and drain to the adjacent evaporation pond. The PCB content of the vactor waste varies depending on where it is taken from, with some of the highest levels near industrial sites. It is imperative that these PCBs are removed before they are washed into the Spokane River, due to already high levels in the water.

In Spokane, the PCB levels in the Spokane River are higher than what is allowed by the water quality standards of both the State of Washington and the Spokane Tribe. PCBs can bioaccumulate in the skin and fatty tissue of fish and members of the Spokane Tribe, whose diet includes fish from the river, have experienced health issues from consuming high amounts of PCBs (Montez & Peterson, 2017). Current methods of PCB-waste removal include transferring the soil waste to another site. This costly method only furthers their spread (2016 Comprehensive Plan to Reduce Polychlorinated Biphenyls, 2016).

Deliverables

The North Central Institute of Science and Technology is requesting funds from the Spokane River Toxic's Task force to fully characterize the factors that contribute to the efficacy of bioremediating PCBs and PAH's at a larger scale.

Funds will be used to pay for comprehensive soil analyses that assess specific PCB congener levels (1668 Protocol and Method 8082A Total PCBs), Total Petroleum Hydrocarbons (NWTPH- Dx), Heavy Metals (Rec 8), and soil microbe profile (Next Generation Sequencing).

Toxics Task Force Proposal

Specific Deliverables will include:

- Data supporting the biodegradation of specific PCB congeners by specific native soil microbes
- Data supporting the soil factors that lead to bioremediation success or failure

Cost

Toxic Task Force Budget			
Item	**Quantity**	**Price**	**Total**
1668 PCB Congener Test	3	$ 700.00	$ 2,100.00
Rec 8 Heavy metal Test	5	$ 85.00	$ 425.00
Method 8082A PCB	13	$ 75.00	$ 975.00
NWTPH-Dx	13	$ 65.00	$ 845.00
Powersoil DNA Extraction Kit	1	$ 400.00	$ 400.00
Oxford Nanopore minION Flow Cells	3	$ 900.00	$ 2,700.00
Oxford Nanopore Library Prep Kit	1	$ 600.00	$ 600.00
Summer Intern wage	2	$ 1,000.00	$ 2,000.00
			$ 10,045.00

Schedule

Month/Year	**Tasks**
March 2022	- Gather 15 gallons of Vactor waste - Homogenize and perform initial analyses - 1668, Rec8, NWTPH-Dx, 8082A, DNA Extraction - Establish Benchtop test bin to ensure biodegradation ability (Time Point A0)
April 2022	- Evaluate Benchtop test bin (Time point A1) - NWTPH-Dx, 8082A and DNA Extraction - Establish 15 gallon outdoor test with initial sediment (Time Point B0)
May 2022	- Gather samples from outdoor test bin (Time point B1) - NWTPH-Dx, 8082A and DNA Extraction - Gather Samples from indoor benchtop test bin (Time point A2)

June 2022	- Gather samples from outdoor test bin (Time point B2)
	  - NWTPH-Dx, 8082A and DNA Extraction
	- Gather Samples from indoor benchtop test bin (Time point A3)
	  - NWTPH-Dx, 8082A and DNA Extraction

July 2022	- Gather samples from outdoor test bin (Time point B3)
	  - NWTPH-Dx, 8082A and DNA Extraction, 1668 Congener Test, Rec8 Test
	- Gather Samples from indoor benchtop test bin (Time point A4)
	  - NWTPH-Dx, 8082A and DNA Extraction

August 2022	- Gather samples from outdoor test bin (Time point B4)
	  - NWTPH-Dx, 8082A and DNA Extraction
	- Gather Samples from indoor benchtop test bin (Time point A5)
	  - NWTPH-Dx, 8082A and DNA Extraction

September 2022	- Gather samples from outdoor test bin (Time point B5)

	- NWTPH-Dx, 8082A and DNA Extraction - Gather Samples from indoor benchtop test bin (Time point A6) - NWTPH-Dx, 8082A and DNA Extraction
October 2022	**-** Gather samples from outdoor test bin (Time point B6) - NWTPH-Dx, 8082A and DNA Extraction, 1668 Congener Test, Rec8 Tests
November 2022	**-** Nanopore sequencing of Extracted DNA
December 2022	**-** Nanopore sequencing analysis of extracted DNA

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