Contamination of Sediment Along the AZHUREV Feed Channel

A Major Qualifying Project submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the degree of Bachelor of Science by:

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Abstract

The AZHUREV constructed wetland started to receive stormwater in 2020, raising concerns that particulate pollution will reach the wetland inlet. We collected sediment samples along the feed channel to analyze the organic matter content, elemental makeup, and presence of polycyclic aromatic hydrocarbons (PAHs) via loss on ignition, accelerated solvent extraction, high-performance liquid chromatography, scanning electron microscopy, and near-infrared spectroscopy. Most particles settled before the inlet likely due to low water flow, providing some water treatment. However, there are concerning PAH levels at the start of the channel. Because of their harmful environmental and health impacts, we propose frequent monitoring and sampling of the feed channel sediment.

Acknowledgements

We would like to give our appreciation to the following people for their contribution to and support of this project: our project advisor, Professor Stephen Kmiotek of Worcester Polytechnic Institute, as well as Dr. Marie-Noëlle Pons and PhD candidate Nicolas Maurice, both from Laboratoire Réactions et Génie des Procédés in Nancy, France. They played a significant role in guiding our research and giving us constructive feedback on our report. Thank you.

Statement on Design

As the culmination of undergraduate studies in Environmental Engineering, WPI requires a Major Qualifying Project with a capstone design element to fulfill the Accreditation Board for Engineering and Technology (ABET) engineering requirement. This requirement states that students must be prepared for engineering practice through a culminating major engineering design experience that incorporates appropriate engineering standards and multiple constraints, and is based on the knowledge and skills acquired in earlier coursework. The design portion of this project involved the design of an in-situ chemical oxidation treatment with the purpose of treating polycyclic aromatic hydrocarbons in sediment from the feed channel of a constructed wetland. The design includes the following criteria:

1. Direct injection of Fenton's reagent into the contaminated sediment using a syringe.
2. Avoidance of dredging and the use of strong oxidants to prevent stir-up and recontamination, disruption of the natural habitat and wildlife, and formation of toxic byproducts.
3. Frequent monitoring and sampling using the same methods outlined in this report after 1-2 days post-treatment.
4. Repeated treatment after 16 weeks if PAH levels are not sufficiently lowered.

Statement on Professional Engineering Licensure

In the career of an environmental engineer in the United States, one important step is obtaining a Professional Engineering (PE) license. Although it is possible to work in the environmental engineering field without a PE license, possessing this certification opens many doors and is generally of great benefit. Holders of PE licenses are the only engineers legally allowed to submit and stamp designs. Without this license, one is limited to working under licensed engineers and will be limited in career progression. Having this license also signifies that one is held to high standards of ethics. The National Society of Professional Engineers creates a strict code of ethics designed to protect the public, and being licensed binds an engineer to that code. This code of ethics is important because it lends credibility for the design work that an engineer does to support human society. Without this code of ethics, the public is at risk of deception and harm by unqualified engineers. Furthermore, holding this license makes an engineer more desirable for hire, either in private consulting practice or by a larger engineering firm, and one is able to establish their own private practice.

To obtain a PE license, one must follow a series of actions. These actions vary by the state in which an engineer desires to practice, however, they all share general similarities. The first step is to graduate with a Bachelor's degree in engineering from a school that has been accredited by the ABET. This means that one has completed a course of education that provides sufficient background to begin an engineering career. The next step is to pass the Fundamentals of Engineering (FE) exam, which is normally taken by students completing their last year of a Bachelor's degree or recent graduates. The FE exam is six hours long and consists of 110 questions, and is offered at various proctoring centers several times each year. Passing this exam allows one to become an "Engineer-in-training", or an apprentice engineer. We personally plan on taking the Environmental Engineering FE exam in the spring/summer to expand our career opportunities. Next, one must gain experience in the field by working under a licensed engineer for at least about 4 years, afterwhich an engineer-in-training can move on to the next step: passing the PE exam. The PE exam consists of 80 questions and lasts for nine hours. Once these steps have been completed, the necessary materials must be submitted to the licensing board in the state that an engineer desires to practice in. The National Council of Examiners for Engineering and Surveying (NCEES) allows for someone who has obtained a PE license in one state to apply for an NCEES record, which consolidates the record of completion of each of these steps and allows for a more streamlined application for a PE license in additional states.

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Glossary

Abbreviation	Term	Definition
ASE	accelerated solvent extraction	timely method of extracting PAHs from environmental mediums
AZHUREV	Aménagement d'une Zone Humide à Reims pour l'Épuration et le Vivant	Development of a Wetland in Reims for Purification and Living
BMPs	best management practices	recommended methods for mitigation of environmental impact
BOD	biological oxygen demand	amount of oxygen consumed by bacteria and other microorganisms while they decompose organic matter under aerobic conditions
DOM	dissolved organic matter	organic material in the form of compounds of C, H, O, N, P, and/or S that may range in size between 0.2 and 0.7 μm, typically 0.45 μm
HPLC	high-performance liquid chromatography	technique in analytical chemistry used to separate, identify, and quantify each component in a mixture
LOI	loss on ignition	change in mass as a result of heating a sample under specified conditions expressed as a weight percentage of the dry mass
LRGP	Laboratoire Réactions et Génie des Procédés	joint unit of the French National Center for Scientific Research and the University of Lorraine consisting of more than 300 people (researchers, teachers, technical and administrative staff, and students)
MAC	maximum allowable concentration	concentration limit of a substance in an environmental medium that the government is willing to tolerate
NIRS	near-infrared spectroscopy	spectroscopy method that uses the near-infrared region of the electromagnetic spectrum (780 to 2500 nm)
PAHs	polycyclic aromatic hydrocarbons	organic compounds consisting of C and H atoms arranged in linked aromatic rings, having low vapor pressures, normally produced from incomplete combustion of organic fuel, and tending to be toxic and harmful to the environment

SEM	scanning electron microscope	microscope that can provide information about the topography and composition of a sample using a focused beam of electrons
TSSs	total suspended solids	inorganic particles larger than 2 μm that drift or float and cause turbidity
VOCs	volatile organic compounds	human-made carbon-containing chemicals used or produced in the manufacture of plants, pharmaceuticals, and refrigerants that have high vapor pressures and low water solubility
WWTP	wastewater treatment plant	facility that collects and treats wastewater before releasing it back into the environment

Chapter 1: Introduction

Water pollution is a problem that society has faced since the beginning of the agricultural era when people first began to live together in communities that grew into populated towns and cities. As society continues to progress with increasingly complicated technologies and practices, the extent and types of pollutants that can contaminate bodies of water multiply. Human and animal waste, dust, debris, and eventually byproducts of industrialization processes have contaminated bodies of water, contributing to widespread problems. The impacts of these pollutants on the environment and human health (two issues that are intrinsically linked since we depend on the quality of our environment for survival) are being observed after-the-fact, so remediation methods are designed in order to correct these impacts. However, there are limitations to each method, and not one method alone can completely restore water quality. Thus, research on the effectiveness of water treatments and the design of new and improved methods must continue until they can meet the demands of a growing population amidst a diminishing clean water supply.

This report investigates one such water treatment method utilized by the city of Reims, France. The AZHUREV CW (Figure 1), which began construction in October 2016, was designed to polish effluent from the Eau du Grand Reims wastewater treatment plant before being discharged to the nearby Vesle river. A stormwater inlet was connected later, and the wetland now receives and polishes water from both sources. The roughly 1 km-long feed channel to the wetland allows for settling of sediment and removal of some pollutants, and the existence of vegetation contributes to filtration and nutrient uptake. Researchers from LRGP, primarily Dr. Marie-Noelle Pons and PhD candidate Nicolas Maurice, have been conducting on-going research and monitoring the quality of the water across the wetland since its completion in July 2017. However, the fate of contaminants in the sediment along the feed channel has yet to be investigated.

The purpose of this project is to sample and analyze sediment along the feed channel and determine how the channel contributes to the removal of certain pollutants before entering the wetland. The analysis methods include LOI, ASE, HPLC, SEM, and NIRS. This research will contribute to informing further remediation efforts at AZHUREV and similar CW projects.

Figure 1. Sunrise at the AZHUREV constructed wetland, January 20, 2022

Chapter 2: Background

2.1 Types of Water Pollutants and Their Impacts

There are various types of pollutants that contaminate water bodies. The first type of pollutant is pathogens, which are viruses, bacteria, fungi, and protozoans that cause diseases in humans, animals, or plants. Some examples of pathogens that may be found in waters are *Salmonella* and *Campylobacter* bacteria that can cause gastroenteritis as well as the hepatitis A virus. Major sources of pathogens are biosolids, on-site treatment systems, untreated or partially treated human wastewater, and animal feeding operations (Gerba & Smith, 2005). Pathogens from these sources can enter waters via stormwater runoff, wildlife and waterfowl, illegally discharged wastewater, and on-site waste disposal (Peconic Estuary Partnership, 2017). Pathogens are a major concern because people can become easily infected through direct contact with or ingestion of contaminated water.

The pollutants that represent the most visible indicator of water quality are called TSSs. TTSs are usually particles that are larger than 2 μm in size and constitute material that drifts or floats such as sediment, silt, sand, plankton, and algae. They reduce water clarity by creating an opaque, cloudy, or muddy appearance. Heavier TSSs can settle to the bottom over a period

of time, whereas remaining particles (called colloidal or non-settleable solids) are too small or too light to settle. TSSs come from soil erosion of stream banks, runoff, discharges, stirred bottom sediments, and algal blooms. Land use and development like construction, logging, and mining contribute to increased TSSs concentrations by exposing soil and decreasing vegetation. High levels of TSSs will increase water temperatures due to the particles absorbing heat from solar radiation. This, in turn, causes decreased dissolved oxygen levels since warmer water cannot hold as much dissolved oxygen as colder water. TSSs can also inhibit photosynthesis by blocking light. With these environmental conditions (low oxygen and little sunlight), the probability of plant survival drops exponentially. Afterward, the decomposition of dead plants causes DO levels to drop even lower. TSSs can reduce habitat quality for fish and other aquatic life, as well, by reducing light penetration and obscuring their vision, which reduces their ability to find food or shallowing the body of water as they settle, which smothers sources of food. TSSs can act as a mode of transportation for other pollutants, which creates potential for widespread pollution of different types (Fondriest Environmental, 2014).

Contrasting to TSSs, DOM is organic material in the form of compounds of C, H, O, N, P, and/or S that is typically smaller than 0.45 μm. These nutrients provide energy and serve as building blocks for organismal growth. They are carbohydrates, lipids, amino acids, nucleotides, and any other compounds involved in growth and decomposition processes. For most systems, carbon compounds dominate, constituting greater than 50% of the DOM pool. A concern with DOM is that it is susceptible to direct photo-degradation in the presence of sunlight, resulting in the direct release of CO_2 and small organic materials, thereby promoting bacterial growth. Additionally, it has the ability to interact with other organic contaminants, acting as binding sites for less soluble organic compounds. Furthermore, if a body of water has a high DOM content, excess chlorination treatment for drinking water purposes can result in the formation of halogenated compounds with carcinogenic properties (Findlay & Parr, 2017).

Trace metals are the next type of pollutant. Some common trace metal pollutants in treated wastewater are zinc and copper. Metals are non-biodegradable, meaning they cannot be broken down into less harmful components in the environment. Chronic low exposures to heavy metals can have serious health effects. Some metals like manganese, iron, copper, and zinc are essential micronutrients, but they can be poisonous in excess. Another worry with trace metals is that they bioaccumulate (assimilate gradually) in tissues. Ionic forms of metals are even more toxic than their elemental counterparts because they can form toxic oxy-radicals with other ions, having the ability to cause serious cellular damage. Small animals are usually the most sensitive to bioaccumulation. For these reasons, bodies of water contaminated with trace metals including mining wastewater and some natural waters are often acidic and threaten aquatic life (Lenntech, n.d.).

Another type of pollutant that results from human activities are micropollutants, which contaminate waters in trace quantities at or below one microgram per liter. Micropollutants include VOCs, microplastics, pharmaceuticals, pesticides, and PAHs. PAHs will be discussed in depth due to its recent emergence and common occurrence in the environment today (Cornell College of Agriculture and Life Sciences, n.d.). PAHs are defined as organic compounds consisting of only carbon and hydrogen atoms and characterized by high melting and boiling points, low vapor pressure, and very low aqueous solubility with varied toxicity and structures. They are composed of two or more benzene rings bonded in linear, cluster, or angular arrangements. Smaller PAHs with two or three benzene rings persist in the atmosphere

as vapor particles. Vapor PAHs that are not completely insoluble can dissolve into water bodies via wet deposition. Larger PAHs frequently persist as solid particles, which can contaminate sediment via adsorption onto mobile colloids, increasing their mobility and bioavailability. PAHs are generated primarily during incomplete combustion of organic materials such as coal, oil, petrol, and wood. PAHs are produced chemically for use in numerous industries and as intermediaries in the manufacture of pharmaceuticals, agriculture products, photographic products, thermosetting plastics, lubricating materials, asphalt, and roofing tar. PAHs are also produced biologically as they are formed during degradation of organic waste by certain plants and bacteria. The most common source of PAHs, however, is motor vehicle exhaust (Abdel-Shafy & Mansour, 2016).

Over time, there has been a vast accumulation of small releases of gas into the atmosphere, generating a cause for concern since PAHs can be absorbed via inhalation, dermal contact, ingestion, or plant uptake. In areas near industrial activity or highways, contamination of vegetation by PAHs can reach ten-fold more than in rural areas. Some of the major routes of exposure to PAHs are drinking water and eating foods contaminated with micropollutants and breathing ambient and indoor air, all of which occur on a regular basis. Although their ability to induce short-term health effects is unclear, a long-term concern with PAHs is that they bioaccumulate; there are detectable levels of PAHs in almost all internal organs of marine mammals with the tendency to localize in body fat due to its high lipid solubility (Abdel-Shafy & Mansour, 2016; Baali & Yahyaoui, 2019). For this reason, concentrations of PAHs in fish are expected to be much higher than in the environment from which they were taken from. PAHs pose significant risk to the health of aquatic life and ecosystems (Baali & Yahyaoui). Some PAHs also have the ability to bind to cellular proteins and DNA, disrupting biochemical processes and causing cell damage, leading to mutations, developmental malformations, tumors, and cancer (Abdel-Shafy & Mansour, 2016). These PAHs are classified as carcinogens to humans by the International Agency for Research on Cancer (IARC) because there is an increased risk of various cancer types (e.g. skin, lung, and bladder) upon PAH exposure (Zhang et al., 2019). PAHs can be degraded by microbes into less complex substances or bioremediated into less hazardous forms, and they can be collected on filters or extracted by solvents. However, there is a research gap for characterization patterns of PAH removal from water bodies (Abdel-Shafy & Mansour, 2016).

2.2 Wastewater Characteristics

Wastewater can be defined as water that has been previously used for industrial, commercial, or household purposes. Some common examples of wastewater sources include cleaning, sewage, cooking, cooling, or hydroelectric production. These uses of water can dramatically change the composition of water, adding many types of pollution, including but not necessarily limited to those described above.

Excessive nutrient load in wastewater discharge can lead to growth of harmful vegetation species. Some of these can be toxic to humans and other animals. In addition, excess vegetation can choke out existing biodiversity in bodies of water (Droste & Gehr, 2019)

Because of the high pollution load that is present in most wastewater, many cities and towns collect and treat wastewater before releasing it back into the environment. However, even treated wastewater can contain pollutants, including pharmaceuticals and PAHs in trace amounts. However, they are being produced and subsequently dispensed into wastewater streams at increasing levels. This growing production, as well as phenomena such as

bioaccumulation, present a growing threat to the environment. As such, focus has recently increased on improving treatment processes to better remove these pollutants (Piai et al., 2019).

2.3 Stormwater Characteristics

Stormwater consists of water from a precipitation event. When this precipitation takes the form of snow or ice, the stormwater consists of the following snowmelt. When stormwater reaches the surface, some water will directly infiltrate and enter the groundwater supply. However, during heavy rainfall, the soil will become saturated and be unable to absorb any additional water, causing the remaining stormwater to pool and flow downhill towards the nearest body of water. When the surface is impermeable, such as roofs and pavement, stormwater can not infiltrate and all will run off. Urban design includes the creation of channels to carry precipitation out of the city to avoid pooling and flooding. Because this project focuses on the fate of stormwater and wastewater in the city of Reims, the stormwater discussed in this context consists primarily of urban runoff. As such, the terms “stormwater”, “urban runoff” and “runoff” will be used interchangeably in this discussion.

There are three major categories of urban runoff: partially sealed surfaces such as green infrastructure and porous pavement, impermeable roof surfaces, and impermeable road surfaces, including other locations of impermeable pavement such as sidewalks and parking areas (Göbel et al., 2007). Each of these categories contributes pollution to urban runoff, and the types of pollution vary based on the characteristics of the surface. Stormwater pollutants can be a wide variety of compounds, but the most commonly discussed are nutrients (including nitrogen and phosphorus), trace metals, and organic pollutants including PAHs. Runoff pollution enters stormwater flows, generally, from two sources of atmospheric deposition: dry deposition and wet deposition (Müller et al., 2020; Gobel et al., 2007). Dry deposition consists of airborne pollutants that settle and stick to surfaces. When precipitation occurs, pollutants are washed off of those surfaces and are either carried by the runoff flow (in the case of larger particles and sediments), or directly dissolved into the water (in the case of water soluble compounds). Wet deposition occurs when pollution in the atmosphere directly attaches to water molecules before or during a precipitation event. When the water reaches the surface, these pollutants are joined by those originating from dry deposition, and all pollutants are carried to the runoff destination. Generally, stormwater is either collected and transported to a nearby body of water, or is locally disposed of by infiltration and percolation (Urbonas et al., 1993). As the runoff continues on its path towards the destination, additional pollutants can be picked up from drainage systems and flow pathways, both by adsorption and wash of dry pollution on the surfaces of those pathways and by movement of previously still water that had collected in drains, puddles, pools, and ditches. This creates an increasing pollution load, and the stormwater at its destination will often be more polluted than the stormwater at its point of contact with the ground. Illegal disposal of wastewater into stormwater drains adds additional pollutants (Muller et al., 2020; Urbonas et al., 1993). Without treatment, stormwater can present a large danger to the environment.

Stormwater carries pollutants and sediments to a body of water. Some of these pollutants are water soluble and dissolved in the stormwater itself, but many take the form of TSSs and the compounds which are adsorbed to the surface of those solid particles. To remove TSSs and the associated pollutants, many treatment designs focus on facilitating settling and sedimentation. The settling behavior of TSSs depends on the characteristics of both the

stormwater and the pollutants, such as the density, size, and settling velocity of the solids and the flow rate and turbulence of the stormwater. Heavier and larger particles tend to settle more quickly than smaller particles, and so are easier to remove. Turbulent water and high flow can keep fine particles in suspension longer, and as such carry those particles further. Because of these factors, pollutants which adsorb to smaller particles will be more difficult to remove from stormwater, and pose a greater challenge when designing treatment and removal methods (Urbonas et al., 1993).

2.4 Grand Reims Wastewater Treatment Plant

The Grand Reims WWTP consists of several steps. The wastewater enters the facility and is first sent through screens to remove large solids. Oil and grease are removed by use of sand traps. Then, the water enters a biological treatment basin, where it is cycled through aerated and anaerobic pools to quicken the degradation of biological material and to allow sludge to clump together. The water is then sent to large round clarification basins where the sludge settles out of the water. Finally, iron chloride is added to remove phosphorus and the effluent is sent through sand filters. According to the Grand Reims WWTP website, over a period of 365 days each of seven different pollutants that are monitored are removed with at least 89% efficiency (Les Amis de Clairmarais, 2016). However, some problematic compounds remain. To further polish the effluent from the plant, treat stormwater, and increase biodiversity in the area, a CW was recently built downstream from the WWTP.

2.5 The AZHUREV Constructed Wetland Project

A CW is an area designed and built by humans that attempts to duplicate the physical, chemical, and biological processes occurring in natural wetland ecosystems in which the combined terrestrial-aquatic environment and diverse vegetation and wildlife interact to improve water quality. Research has shown that CWs are effective in removing pollutants such as BOD, suspended solids, nitrogen, phosphorus, hydrocarbons, and metals and are therefore widely used in polishing treated wastewater, especially for meeting tougher national or European water quality standards. Other benefits of CWs are that they are less expensive than other treatment options, they require less energy, supplies, on-site labor, and maintenance, and they can double as public attractions. This technology demonstrates that man-made projects can exist harmoniously with nature to the advantage of both humans and the environment; however, treatment possibilities of CWs need to be better understood with further research (Gelt, 1997).

The AZHUREV CW in Reims consists of three 20,000-m^2 rectangular basins, each with a different depth distribution, initially planted with different densities of *P. australis*, *S. lacustris*, and *G. maxima* (Pons et al., 2019). Water is routed to these basins via an inlet channel roughly one kilometer in length which carries both effluent from the WWTP and stormwater. The CW polishes reclaimed wastewater and stormwater via particle settling along the length of the basins, nutrient uptake from the plants, and microbial biodegradation before discharge into the Vesle river.

The project started in 2011 in response to a request by the Ministry of Ecology. Upon approval in 2012, preliminary planning and design began, including investigating the nature of the soil in the area, the slope of the ground, the social impact of construction, and the local fauna. Construction began in October 2016, and reclaimed wastewater was introduced to the basins in July 2017 when monitoring of the wetland by LRGP began. Monitoring was initially scheduled through 2018 but was extended upon request by the Reims Metropole and is currently expected

to continue through 2024. Although the WWTP removes carbon, nitrogen, and phosphorus, data reveals that some remain in the treated wastewater. In 2018, invasive vegetation (mainly duckweed and *Ceratophyllum* sp.) was removed, which loosened some sediment and released heavy metals into the water, indicating that the sediment was previously contaminated. Stormwater treated with grit removal started to feed into the CW in 2020, after which valves operated to control inflow from both water sources. Figure 2 below shows a map of the AZHUREV CW and the feed channel.

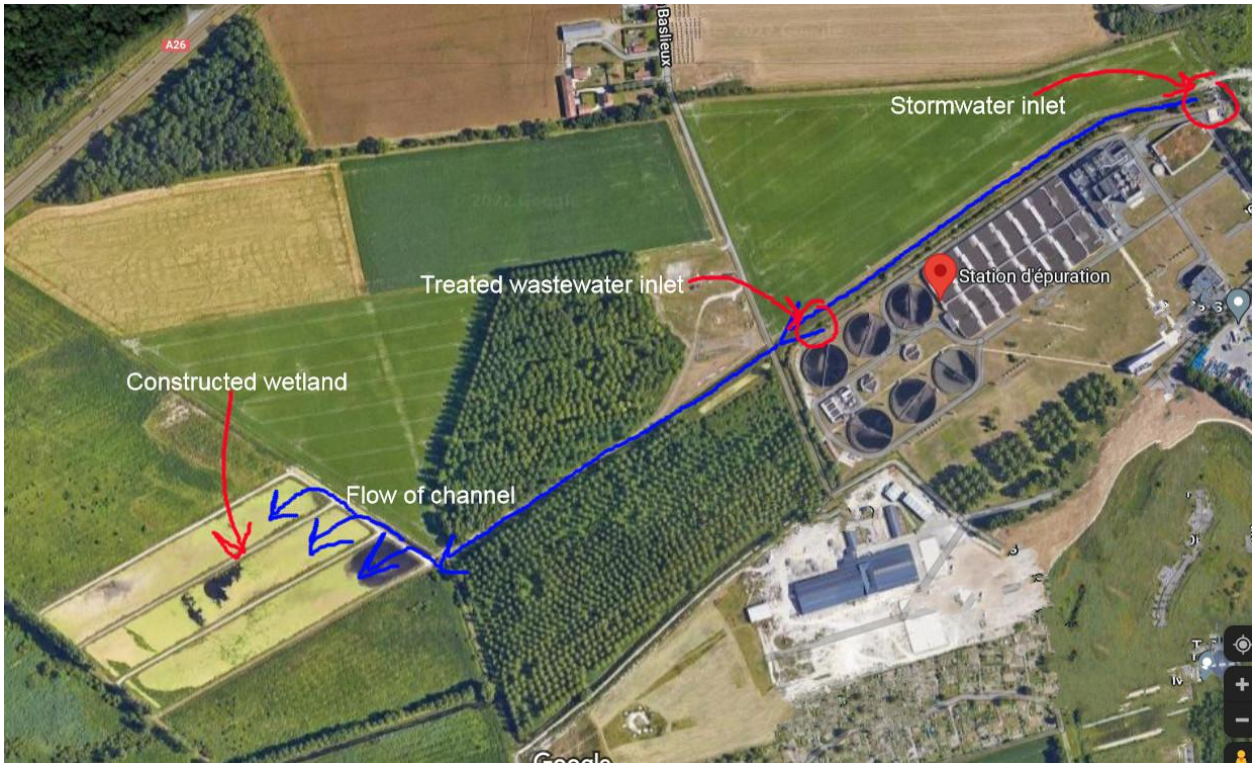

Figure 2. Map showing the Grand Reims WWTP, the feed channel, and the AZHUREV CW (Google Maps)

Since stormwater can be contaminated with micropollutants such as metals, PAHs, and pesticides attached to settled particles in the feed channel, it is possible that the sediment in the feed channel is enriched with these compounds. Therefore, the current objective of the project is to characterize the sediment along the channel by measuring the content of organic matter, elements and trace metals, and PAHs. Because the flow through the channel is generally low, we predicted that most of these particles settled upstream.

Chapter 3: Methods

3.1 Sample Collection

AZHUREV sediment collection took place on January 19, 2022. We started downstream at the inlet to the CW basins and proceeded in the upstream direction because it

would prevent disturbed and resuspended sediment from affecting subsequent samples. First, we placed stakes along the feed channel approximately 50 m apart, measuring the distance using surveying tape. We placed a total of 21 stakes with the last stake right before the stormwater inlet, making the total examined length of the feed channel approximately 1.05 km. At each stake location, we recorded the latitude and longitude coordinates from Apple Maps (Appendix A, Table A1). The wastewater effluent joined the channel in-between stakes 10 and 11. Pictures of the channel are shown in Figure 3. Green vegetation can be observed at the downstream end of the channel, and the water is visibly clearer.

Figure 3. Segments of the feed channel
Left. Upstream from the wastewater entrance. **Right.** Close to the wetland inlet

Then, at each stake location starting at 1, we collected sediment cores using 60-mL syringes with open ends (Figure 4). We put on waders and wet gloves for protection against the cold water. To collect the sample, we pushed the syringe into the sediment while simultaneously pulling the plunger.

Figure 4. Sample sediment core collected from location 3

Two samples were collected at location 1 to allow us to practice the collection method; these samples were labeled 1A and 1B. The stream bed was rocky at some locations, making collection difficult and resulting in smaller relative sample sizes. We observed that the vegetation was thick near the beginning and end of the channel, so we predict that they contribute a filtration effect. Dr. Pons went on a second trip to AZHUREV to collect additional samples at three new locations along the feed channel. The first (R68-1) was close to location 1, R68-2 was approximately in the middle of the channel, and R68-3 was further upstream. Three samples were taken at each of these locations and denoted A, B, and C.

There are some errors and uncertainties with the sample collection method. The act of stepping into the channel, as well as pushing the syringe into the sediment to collect the sample, naturally disturbed the sediment layer. The sediment was further disturbed in cases where vegetation had to be pushed out of the way before sample collection, or when the first attempt at collection was unsuccessful, and the process had to be repeated. These disturbances would cause the most recently deposited sediment to rise up into the water. Because of this, the collected samples may not be representative of the most recently deposited sediment. In addition, the sample collection method was designed to preserve sediment cores as much as possible, so as to include both recent and older sediment layers. However, because of the difficulty in collecting these samples, some syringes were not exactly perpendicular to the surface of the sediment. This, along with the varying sample sizes, would mean that the oldest sediment contained in some samples is actually much newer than the oldest sediment contained in others. In addition, we do not know the rate at which the sediment settles, or the exact flow rate of the channel, as these would vary with environmental conditions. This makes it impossible to draw conclusions about the exact age of the sediment in the samples collected.

3.2 Preparation of Spiked Control Samples

In order to provide a basis for comparison, two samples were spiked with Dr. Ehrenstorfer PAH Mix-9 (Appendix C), which is a mixture of 16 PAH compounds. One sample consisted of soil collected on December 7, 2017 from Reims outside of the channel but near the inlet to the CW basins, and the second was regular potting soil (Figure 5). The spiking procedure we used to prepare the samples is below.

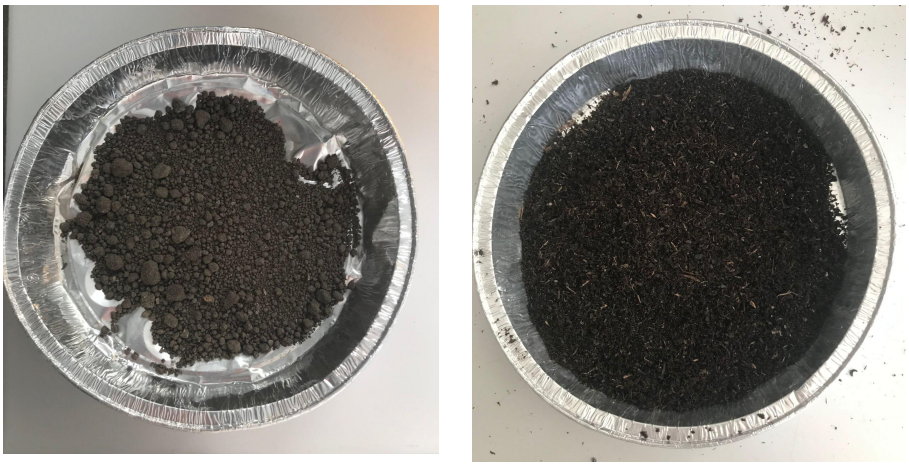

Figure 5. Spiked control samples
Left. Soil from Reims prior to grinding. **Right.** Potting soil after sieving

Sieve the potting soil to remove any large pieces such as rocks, sticks, and chalk using a 2-mm sieve (Figure 6). Leave the sample to dry completely. Grind the Reims soil using a mortar and pestle. Measure 2 g of each soil using a mass balance. Add 1 mL of PAH Mix-9 to each sample in a shallow glass dish and carefully mix using a spatula. Leave the samples under a fume hood to let the solvent fully evaporate (approximately 2 days). Carefully transfer each sample into separate and labeled screw-on cap tubes for later experiments.

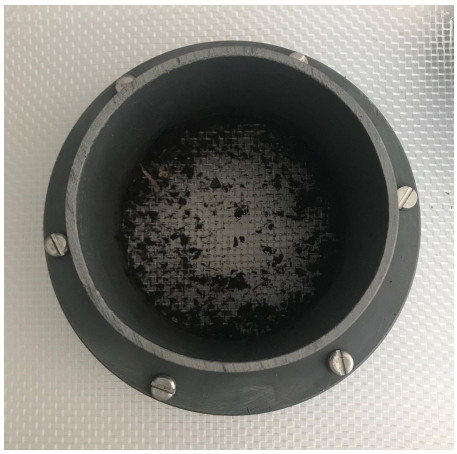

Figure 6. Sieve used to remove large particles from soil

There are some uncertainties associated with the preparation of the control samples. The first is that the PAH mixture was found in storage with slightly more than 2 mL remaining, limiting our spiking concentration. The mixture expired in March 2007, and there was no information about how the quality of this mixture degrades over a long period of time. We assumed that the expiration date did not negatively impact the results since the ASE machine was able to detect the presence of PAHs in the spiked samples. Another uncertainty with the

materials is the preservation of the Reims soil from 2017, which was stored in a sealed bag at 4℃ in the dark. During the preparation, cross-contamination of the soil while grinding and loss of mass while sifting, transferring, and spiking the soil are possible uncertainties as well.

3.3 Preparation of AZHUREV Samples

Lay the samples onto individual aluminum trays. For sediment cores larger than about 2 cm tall, separate them into two trays with the underlayer labeled “u”. This was done because the smallest sample was approximately 2 cm in depth, and the top layer of soil is expected to be more contaminated. The samples must be placed in a freezer for a minimum of 24 hours. We placed our samples in the freezer for four days. Next, they were lyophilized, nine samples at a time, in a freeze dryer with a low-pressure vacuum for an additional two days (Figure 7). This is an initial drying process intended to remove any moisture from the samples for use in later experiments.

Figure 7. Alpha 1-2 LDplus freeze dryer

Once samples have lyophilized, grind the soil to a fine texture using a mortar and pestle. Carefully remove any large pieces of rocks, vegetation, or other non-soil materials using a tweezer. Sieve each sample and pour through a funnel into separate and labeled screw-on cap tubes for later use.

There was wide variability between the volumes of the sediment cores, ranging from about 2 cm to 6 cm in depth, so some sediment cores taken from the feed channel were not deep enough to be separated into a top layer and an underlayer. This leaves some gaps in our data, leading to incomparability between samples. Smaller sediment samples would have been the most sensitive to cross-contamination while grinding using the same mortar and pestle or transferring using the same funnel. Furthermore, larger pieces of chalk in some of the samples could have been removed via sieving instead of being kept and ground as part of the sample,

leading to less calcium than the actual quantity. The drying process was extensive enough that we are certain there was close to no moisture in the samples, and the indoor humidity was not significant enough to cause moisture to accumulate.

3.4 Loss by Ignition

The loss by ignition method allows us to estimate the amount of organic matter in sediment samples by oxidizing organic matter at a very high-temperature. The AZHUREV samples as well as the "clean" (not spiked with PAH Mix-9) samples of the 2017 Reims soil and potting soil were tested with loss by ignition. The experiment procedure is outlined below.

Acquire ceramic pots and number the bottoms with a pencil. Keep note of these numbers and their corresponding samples. Using a precision scale, weigh each sample pot and record the mass. Add between 0.5 to 1.0 g of the corresponding sample, depending on how much sediment is available. Weigh and record the mass of the pot plus the sediment. The samples are now ready for ignition, as shown in Figure 8 below.

Figure 8. Samples ready for loss by ignition

Place the samples in the oven (Figure 9). Set the maximum temperature to 525℃ for a heating period of 2 hours, a constant temperature period of 2 hours, and an overnight cooling period. The starting temperature should be around ambient temperature (about 15℃). Close the hood, turn on the ventilation, and begin the cycle.

Figure 9. Nabertherm oven used for loss by ignition

After the oven is completely cooled, turn off the ventilation and take the samples out. Measure the mass of each sample pot containing ash. The difference in mass before and after ignition is used to calculate the LOI. Carefully transfer the ashes into small falcon tubes using a spatula for later analysis with the SEM (Figure 10). Mass values for this experiment are shown in Appendix A, Table A2.

Figure 10. Sample ashes in falcon tubes

A human error that could have occurred for this method was incorrectly matching a pot number to a sample number. This could have matched mass values to the wrong sediment sample, leading to faulty data. However, this is unlikely to have bypassed two people. The

precision scale that we used gave high accuracy mass values with four decimal points, and the automated ignition oven was operated at the same settings for each run.

3.5 PAH Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) is a relatively new technology that provides a more convenient, faster, and less solvent intensive method of extracting PAHs from environmental mediums. The machine pumps solvent into an extraction cell containing a sample. The contents of the cell are then brought to an elevated temperature and pressure. The extracted mixture is transferred from the heated cell to a glass collection vial for analysis. The entire process is automated and performed in minutes (Dionex, 2011). The detailed procedure is outlined below.

Prepare cells for accelerated solvent extraction (ASE) as follows (Figure 11). Screw the bottom cap onto the extraction cell. Load the bottom of the cell with 3 mm-diameter spherical glass beads at a depth of approximately 1 cm. Carefully insert a circular microfiber filter so that it lies flat on top of the glass bead layer. Measure and add approximately 1 g of florisil and 1 g of anhydrous sodium sulfate. Insert a second filter on top of the powder layer. Add 2-4 g of sediment, depending on how much is available for testing. Measure and record the mass of the sediment. Keep note of which cell corresponds to which sample. Insert the last filter on top of the soil layer. Add one last layer of glass beads at a depth of approximately 1 cm. Screw the top cap of the cell by hand. Make sure that it is completely tightened.

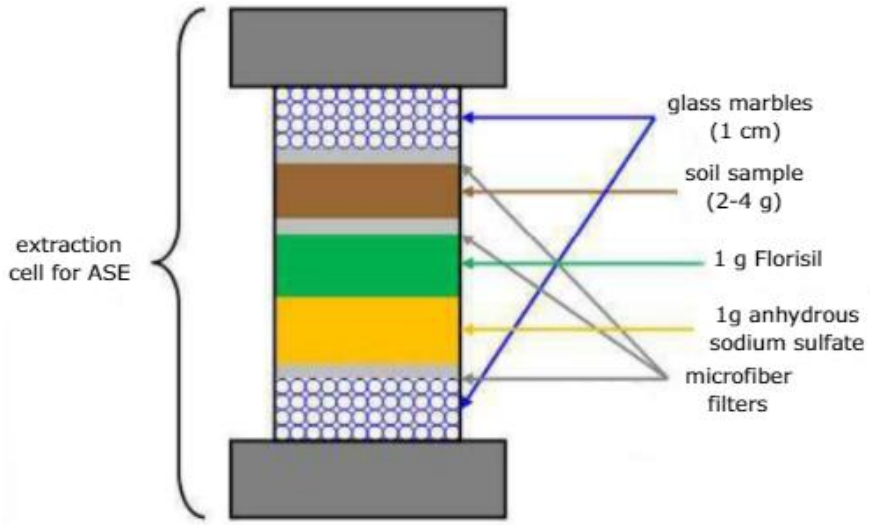

Figure 11. Layering the ASE extraction cell contents

Next, load the cells and the vials into the ASE machine, as shown in Figure 12 below.

Figure 12. Cells and vials ready for extraction in the Dionex ASE 350

In the designated receiving slots, load the corresponding glass vials. Label each vial correctly. Configure the ASE settings as follows:

- Temperature: 100℃
- Heat time: 5 minutes
- Static time: 5 minutes
- Cycles: 1
- Rinse volume: 60%
- Purge: 60 seconds
- Solvent A: Acetone
- Solvent B: Dichloromethane

Turn on the flow of nitrogen to the machine, then start the extraction process. The extraction takes about 15 minutes for each cell. When the extraction has finished and the equipment has cooled, remove the cells and the vials from the machine (Figure 13). Measure and record the mass of the vials and store them in the refrigerator.

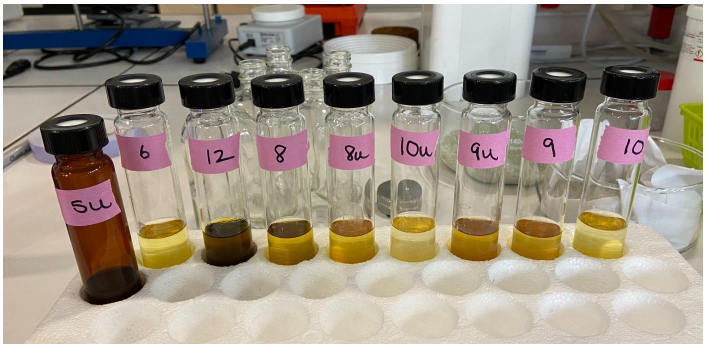

Figure 13. Sample vials containing solvent and extracted PAHs

When emptying the cells, remove the glass beads and wash them thoroughly with water, and discard soil, microfiber filters, florisil, and sodium sulfate accordingly. Clean the empty cells in the dishwasher and allow them to dry for reuse.

During the process of preparing the cells for extraction, the layers were not always consistently the same depth or perfectly separated. For example, the glass beads were slightly variable in size, and the number of beads per cell was not counted. Also, pressing the microfiber filter into a cell sometimes displaced some of the florisil and sodium sulfate powder on top of the filter. These slight differences combined may have uniquely affected the extraction process for each cell. Therefore, it is important to be careful when loading the cells with the contents to prevent mixing layers and to keep factors as similar as possible. Furthermore, some vials were not massed before and/or after extraction, or their caps had to be switched out and were not re-weighed, so for these samples, it is unknown how much of the solvent was injected into the corresponding cells. This is missing data that could have been useful in our analysis of PAHs.

3.6 Scanning Electron Microscopy

An SEM provides information about the sample surface topography and composition. We used SEM technology to determine the elemental composition of the AZHUREV sediment along each point of the feed channel. The components of the microscope are a source of electrons, a column down which electrons travel with electromagnetic lenses, an electron detector, a sample chamber, and a computer to display images. The sample is mounted on a stage in the chamber area, after which both the column and chamber are evacuated by a combination of pumps to establish a low pressure environment. Electrons are produced at the top of the column and accelerated down and passed through lenses to produce a focused beam that hits the sample surface. The position of the beam is controlled by scan coils situated above the objective lens, which allow the beam to scan over the surface. A diagram of the equipment is shown in Figure 14 below.

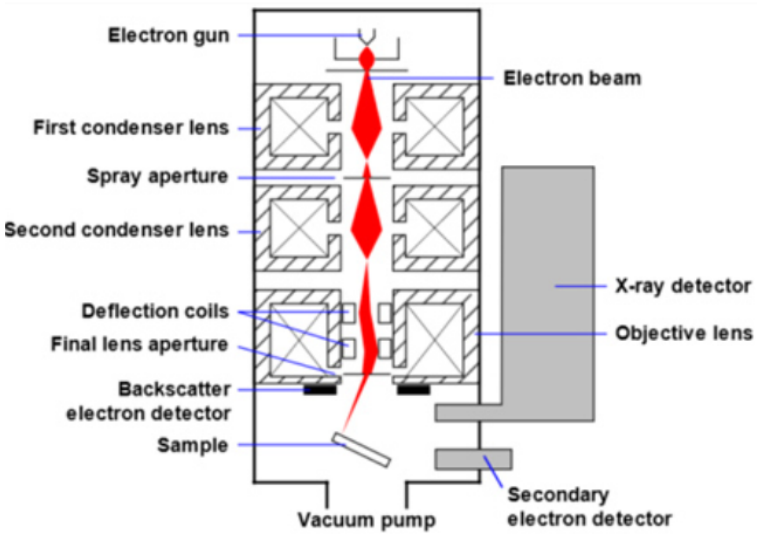

Figure 14. Diagram of an SEM column (Nanoscience, n.d.)

When the beam hits the surface, it penetrates the sample to a depth of a few microns, depending on the set voltage and the density of the sample. As the electrons interact with the sample, the secondary electron detector counts the rays depending on their energy and forms images characteristic of the nature of the atoms. In our analysis, we detected the presence of 14 elements: Na, Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Ni, Zn, and As. The SEM produced results at three spectra and took the averages in atomic percentage (A%) for each element, which we graphed for data analysis. The SEM procedure is detailed as follows.

Acquire a SEM stub for each sample. Apply double-sided carbon tape across the top of the stub. Using a tiny spatula, spread a small amount of sediment across the tape. The sediment used for SEM analysis were the ashes from the loss by ignition procedure, as organic material had been burned off and the elemental makeup of the sediment would be simpler to analyze. Tap any excess sediment off. Repeat for all samples, and make sure to keep note of which stubs correspond to which samples. Place the stubs on a shallow glass dish for easy transport.

Place eight stubs at a time into the ion sputter machine to be covered with a thin layer of gold and palladium. This serves as a pre-treatment to the SEM and should aid in better electron interaction (Figure 15). Set the voltage to 1.2 kV and the pressure to 5 mA and start the machine.

Figure 15. Eight sample stubs in the JEOL Fine Coat JFC-1100 Ion Sputter

After the coating is finished, remove the samples from the ion sputter, fit them into the sample stage, and place them in the SEM chamber (Figure 16). Set the voltage to 20 kV and run the x-ray microanalysis, which should take about 2 minutes per sample. After data collection, allow the SEM to settle to ambient pressure before removing the samples. Keep in mind that samples cannot be reused.

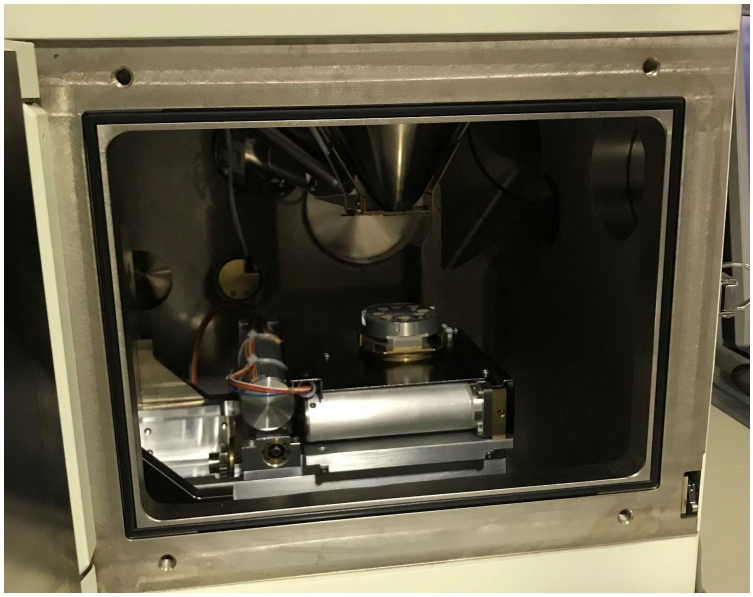

Figure 16. Interior of the JEOL JSM-6490LV SEM containing eight samples

Some of the sediment samples contained larger pieces of chalk. Therefore, the goal of sample preparation was to crush up most of the chalk with the soil during grinding, but there may have been some chalk that was removed during sieving. This would alter the calcium makeup of the sediment to be slightly different than its actual composition, so the calcium results can only serve as close approximations. While preparing the stubs for SEM analysis, the numbers on the stubs could have been matched with the incorrect sample number, though unlikely. Additionally, there was potential for cross-contamination since the same spatula was used to prepare each sample and the sample volumes were extremely small. This uncertainty was minimized by wiping the spatula between every sample. Nevertheless, the SEM produces highly accurate results that can be graphed, compared, and reproduced.

3.7 High-Performance Liquid Chromatography

HPLC is a separation technique used to determine the composition of a sample, usually for the analysis of ions, proteins, and organic molecules. The apparatus consists of a pumping system, an injector, a chromatography column, a detector, and a data collecting device. The pump ensures an adjustable, constant flow to the column, while the injector introduces a narrow stream of sample volume onto the top of the column. The machine that we used specifically consists of the following elements: a bottle of acetonitrile, a bottle of water, a two-way degasser, two piston pumps with a pressure stabilizer and a bypass, a 5 μm Phenoménex Envirosep PP column with dimensions 125 mm by 4.6 mm, and a diode array detector that records a multi-wavelength spectrum (Arnoux, 2009).

The process involves a solid stationary phase (the column) and a liquid mobile phase (the solvent). The technique is based on the principle that some components of the sample take longer than others to pass through the column. The time that each component takes depends on their selective affinity with the mobile phase and the stationary phase. For example, components with a greater affinity with the stationary phase will take longer to pass through the column. The operating principle is shown in Figure 17 below.

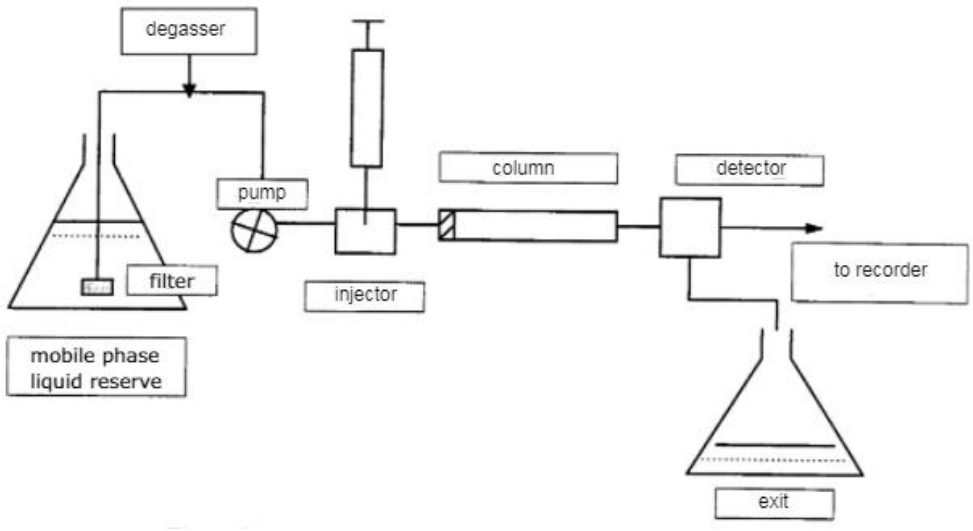

Figure 17. Process diagram of HPLC

HPLC is quicker and more efficient compared to other separation techniques; it delivers highly precise, reproducible, and reliable results in about 30 minutes. Another advantage to the technology is that it requires a small sample size and minimal monitoring with automated equipment (Conduct Science, 2019). We used HPLC with detection by UV-Vis spectroscopy, which is the measurement of spectra produced by matter interacting with ultraviolet radiation of wavelengths between 100 and 400 nm, to identify and quantify the presence of 16 PAHs. They are shown in Table 1 along with their molar masses, retention times, and maximum wavelengths.

Table 1. The 16 US-EPA priority PAHs (LGC Group, n.d.)

PAH	Abbreviation	Molar Mass [g/mol]	Retention Time [min]	Max Wavelength [nm]
Acenaphthene	ACE	154.21	8.1	227
Acenaphthylene	ACY	152.19	6.65	229
Anthracene	ANTH	178.23	10.5	251
Benz[a]anthracene	B[a]A	228.29	15.2	287
Benzo[a]pyrene	B[a]P	252.31	20	296
Benzo[b]fluoranthene	B[b]F	252.31	18	256
Benzo[ghi]perylene	B[ghi]P	276.33	22.25	299
Benzo[k]fluoranthene	B[k]F	252.31	19.2	306
Chrysene	CHRY	228.29	15.8	267
Dibenzo(a,h)anthracene	D[ah]A	278.35	21.8	296
Fluoranthene	FLTH	202.25	11.5	235
Fluorene	FLU	166.22	8.5	205
Indeno[1,2,3-cd]pyrene	IND	276.34	23	249
Naphthalene	NAP	128.17	5.65	220
Phenanthrene	PHEN	178.23	9.5	250
Pyrene	PYR	202.25	12.3	240

To prepare for HPLC, remove the ASE extraction vials from refrigerated storage. Place the vials into the evaporator. The evaporator consists of a water bath at 40℃ with nitrogen air flow (Figure 18). Leave the samples in the evaporator for about 35 minutes, depending on the solvent supply and the rate of evaporation of the solvent. With these conditions, the

acetone-dichloromethane solvent mixture should mostly evaporate. However, make sure to not let the solvent completely evaporate. When the volume is about 2 mL, add 5 mL of acetonitrile to each vial. Continue the process until the original solvent mixture has completely evaporated and acetonitrile has replaced the solvent, an additional 20 minutes.

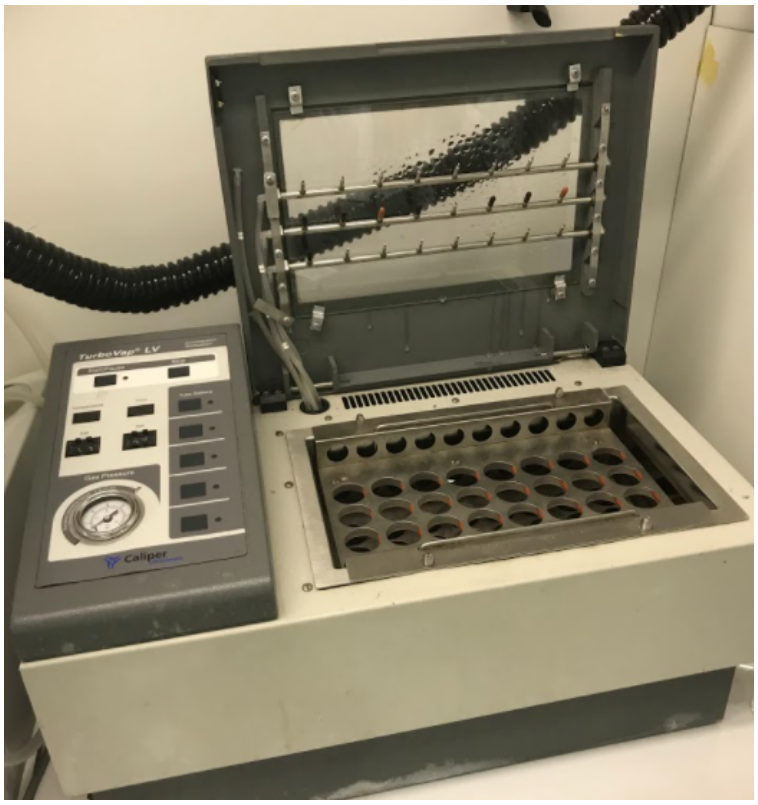

Figure 18. TurboVap LV evaporator

After evaporation, remove the vials and transfer the contents into 2-mL tubes with patch caps using a sterile 2-mL syringe attached to a 26-mm cellulose filter (Figure 19). The samples are now filtered and ready for HPLC.

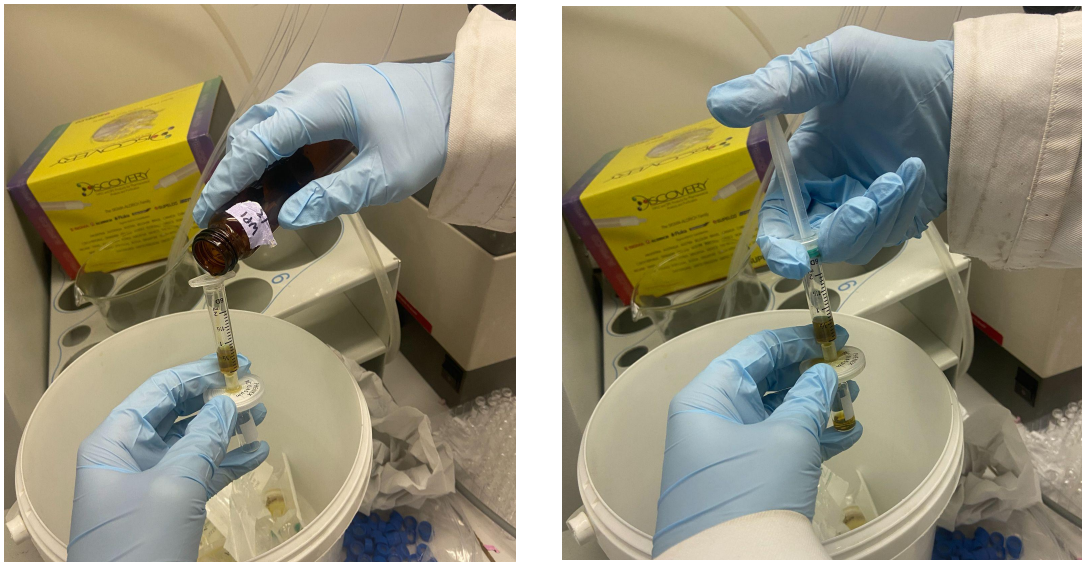

Figure 19. Filtration prep before HPLC
Left. Pouring sample into syringe. **Right.** Pushing sample through filter into vial.

Place the samples in the HPLC machine. Load a sufficient supply of acetonitrile as the solvent. Configure the settings as follows:

- Pump flow rate: 2 mL/min
- Lower limit pressure: 50 bars
- Higher limit pressure: 150 bars
- Acquisition range of the photodiode array: 190-350 nm
- Column oven temperature: 35℃
- Sample temperature: 15℃
- At t = 0 min, 40% acetonitrile
- At t = 25 min, 100% acetonitrile
- At t = 30 min, 100 % acetonitrile
- At t = 35 min, 40% acetonitrile

Numerous errors and uncertainties occurred during this method. Once the procedure was done, some vials had sample volumes much less than the 5 mL of acetonitrile that was added. Most of the samples that we worked with were between 2 and 2.5 mL. Samples 1Au, 6u, and 17 were not even salvaged for HPLC since all of the acetonitrile had presumably evaporated. This introduces variability that has to be considered in our calculations. We believe that some acetonitrile evaporated during the process (even though its boiling point is 82℃) due to the nitrogen flux. Also, the samples were not in the evaporator for the same amount of time and the addition of acetonitrile was based on personal judgment. Some samples were probably ready to be taken out earlier. A recommendation we would make to improve this procedure and to limit these uncertainties is to run the samples for the same amount of time and to add acetonitrile at the same time. We also recommend waiting less time after the addition of acetonitrile and continuously checking the samples every 5 minutes until they are ready to be taken out.

Furthermore, filtration with 0.2 μm polytetrafluorethylene (PTFE) membranes would have allowed for a more precise quantification of PAHs. These membranes are resistant to temperature up to 260℃, are naturally hydrophobic, and can filter extremely small particles. However, a large pressure was necessary to use them, which caused the filters to explode. Instead, cellulose filters were used, which could not provide completely quantitative results. With these errors and uncertainties, the HPLC analysis limited our ability to obtain accurate data and make informative conclusions about the presence of PAHs.

3.8 Near-Infrared Spectroscopy

NIRS is a rapid and non-destructive sampling method similar to UV-Vis spectroscopy that can perform both qualitative and quantitative analyses. However, NIR absorptions require more energy than a fundamental absorption from a chemical bond (AZO Materials, 2021). The method is based on absorptions in the near-infrared region between 780 and 2500 nm that are generated from vibrations by overtones and combinations. Overtones arise from a series of absorptions at multiples of the frequency, or the reciprocal of wavelength. Combinations arise from two or more fundamental absorptions sharing NIR energy. A high number of combinations can be observed based on the number and types of bonds in the molecule. The effects of these absorptions combine to create unique spectra consisting of only a few broad peaks. A fundamental O-H absorption, for example, is different from a fundamental C-H absorption, so their overtones and combination bands will be different (Davies, n.d.).

The benefits of NIRS are that it produces accurate results in under one minute, it measures a range of parameters in a single run, it does not require sample preparation, it does not chemically alter, damage, or destroy samples, allowing them to be reused, and it does not require the use of toxic reagents or solvents, proving to be an environmentally friendly technique (Davies, n.d.). NIRS offers a method to rapidly measure PAHs in soils, allowing one to gain knowledge about their concentration and compositional distribution. Early identification and characterization of PAHs in soils may reduce the costs associated with their future management and possible impacts of the potential spread of pollution . However, because of broad and overlapping bands, there is a difficulty associated with interpreting NIR spectra. In addition, there have been high false positive rates of PAHs reported using NIRS, reflecting the need for further research on its application. Therefore, NIRS is often used in conjunction with other analytical techniques (Okparanma et al.). We used NIRS as another method to analyze the PAH content of the sediment. The procedure to collect spectra results is outlined below.

Turn on the NIR analyzer one hour before the start of the analysis to heat up. Set the analyzer for sampling by fiber optics. The program will prompt you to measure the reflectance of a sample of barium sulfate powder as background spectra. Use a diluted isopropanol-water mixture and delicate task wipes to clean the tip of the handheld probe in between samples. Once you have scanned the barium sulfate, you can measure the reflectance of the sediment sample. During each scan, make sure that the tip is in contact with the sample and put a black cloth over the sample and the probe to avoid light interference. The analyzer probe can be seen in Figure 20 below.

Figure 20. Antaris II FT-NIR Analyzer probe

There are a few uncertainties that could have occurred during data collection, including light interference, cross-contamination, and incomplete contact of the probe with the sample. Starting the scan too early or removing the probe in the sample too early before the machine had completed data collection are also possibilities. However, we believe that we obtained a comprehensive collection of accurate data, especially since the probe completed a total of 16 scans for each sample analyzed, and we actively tried to limit these uncertainties with necessary measures.

Chapter 4: Results

4.1 Decreasing Levels of Organic Matter

The loss by ignition method in which we oxidized organic matter in the samples at a very high temperature allowed us to calculate a scientific estimate of the concentration of organic matter in each sample, as shown in Appendix B, Table B1. We graphed these values from location 21 where the stormwater inlet is to location 1A near the CW inlet to display how the concentration changed along the direction of flow. We also graphed these values against the values for the clean potting and Reims soils for comparison (Figure 21).

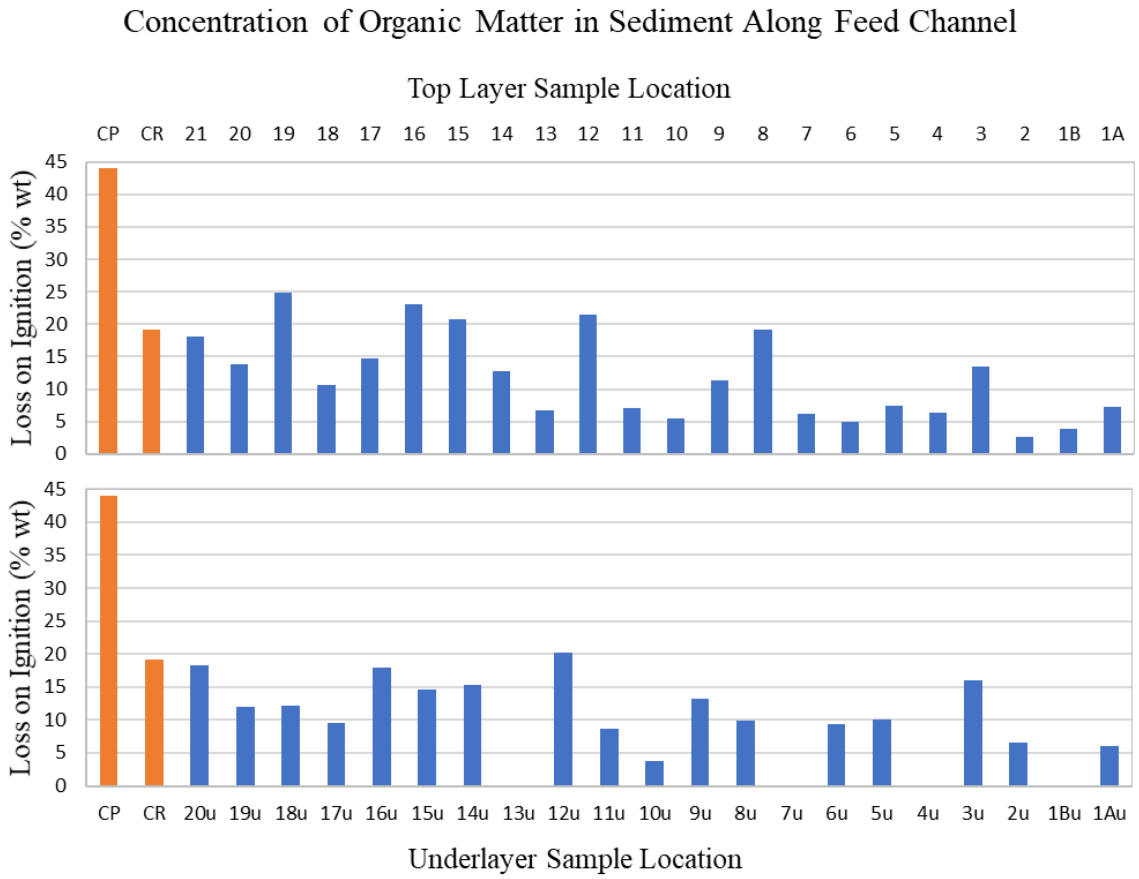

Figure 21. Concentration of organic matter represented as % LOI values at each sample location in blue compared to the control samples in orange

The first noticeable thing is that all of the samples taken from AZHUREV as well as the clean Reims soil have an organic matter concentration much less than that for the clean potting soil. It makes sense that the potting soil contains a higher level of minerals and nutrients such as nitrogen, phosphorus, and sulfur because they are essential for crop growth.

Another deduction from Figure 21 is that the organic matter content generally decreases along the feed channel from the start of the stormwater inlet towards the wetland inlet. The LOI starts at a value of 18.1% at sample 21 and ends at 7.2% at sample 1A, or 3.8% at sample 1B taken at the same location. Evidently, the concentration drops gradually as a result of settling. The values for the AZHUREV samples are comparable to the LOI value for the clean Reims soil. Ultimately, the concentration of organic matter in the feed channel sediment does not reach outstanding levels.

4.2 Benign Elemental Makeup with Expected Trends

The SEM analysis allowed us to analyze the elemental composition of the sediment samples. We investigated the presence of As, Cl, Mn, Ni, K, Na, Zn, Mg, S, P, Fe, Al, Si, and Ca. We graphed results in a stacked column chart style to compare the atom percent of each element

at each sample location. We found that trace elements S, Mg, Zn, Na, Mn, K, and As were each less than 2.5% in all samples and showed little variability between samples. Their concentrations are insignificant compared to the entire makeup of the samples, so this finding presents nothing concerning. Silicon and aluminum make up a significant portion of the samples, but they are normal components of soil and are not of our concern in terms of contamination.

When graphing the total composition, we saw that Ca made up the majority of each sample, around half of the total composition (Figure 22). This makes sense because the calcium comes from the chalk that was present in each sample.

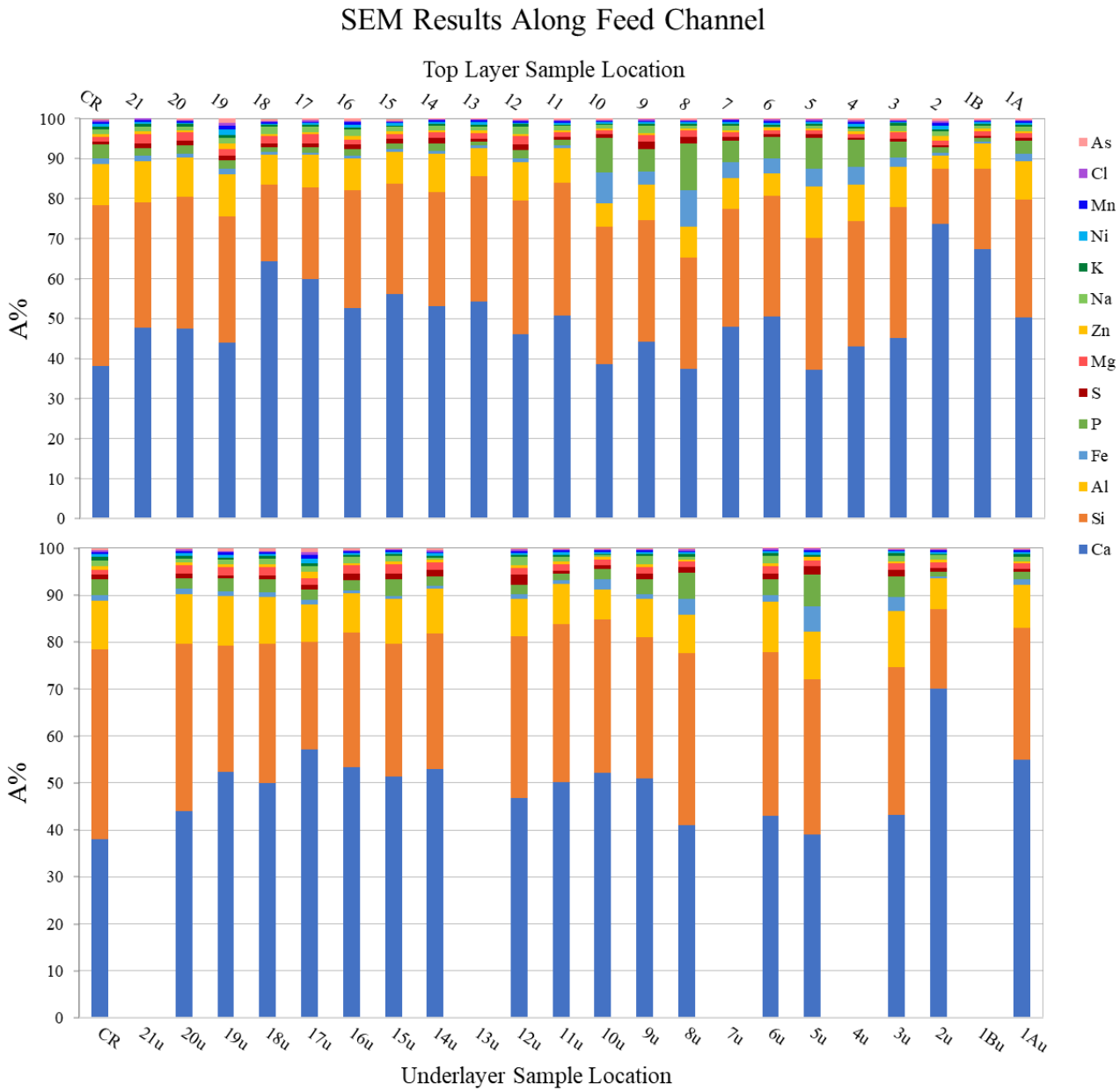

Figure 22. Atom percentages of each element at each sample location

Next, we graphed the results for Fe and P individually, as shown in Figures 23 and 24 below.

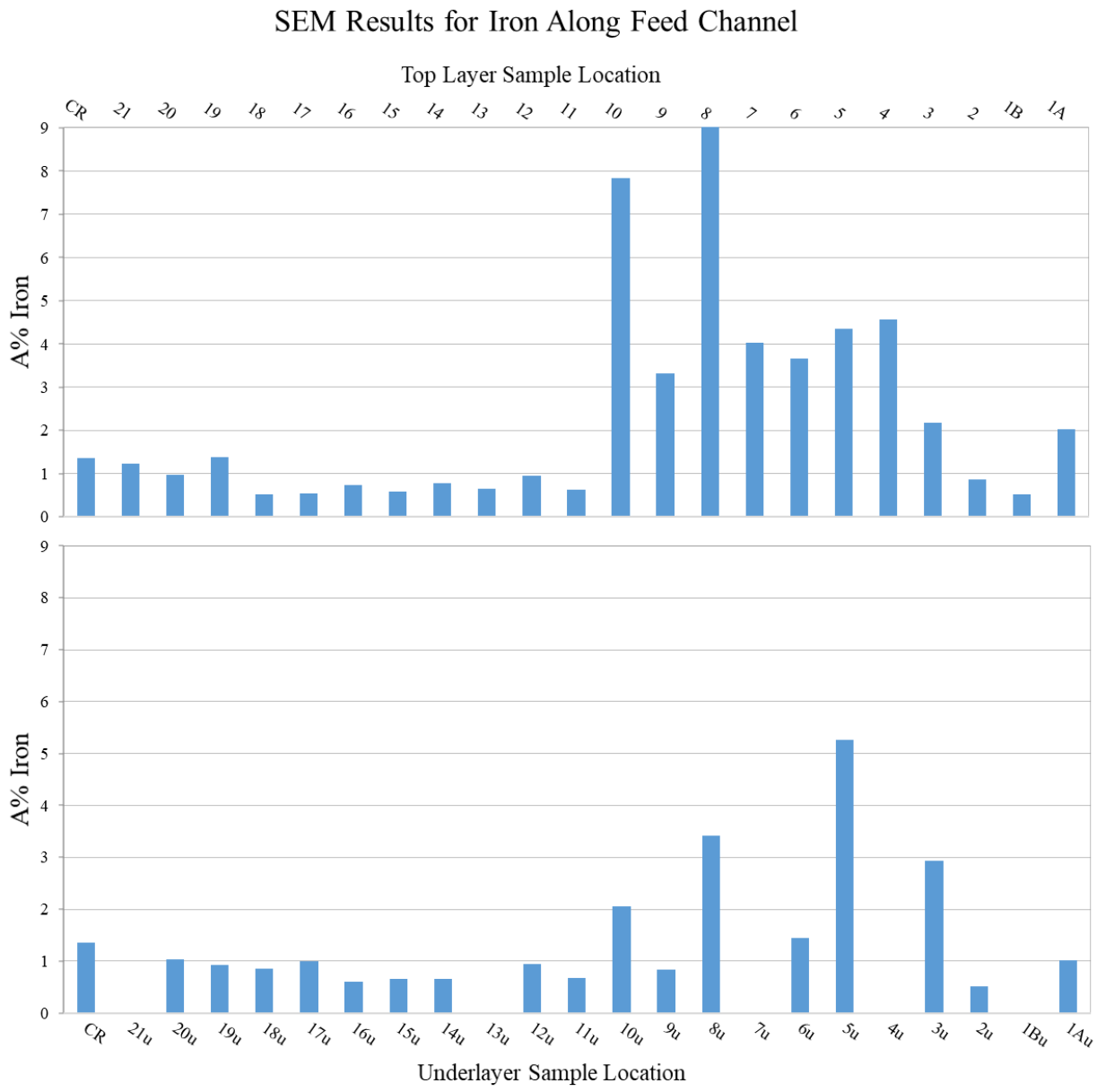

Figure 23. Atom percentages of iron at each sample location

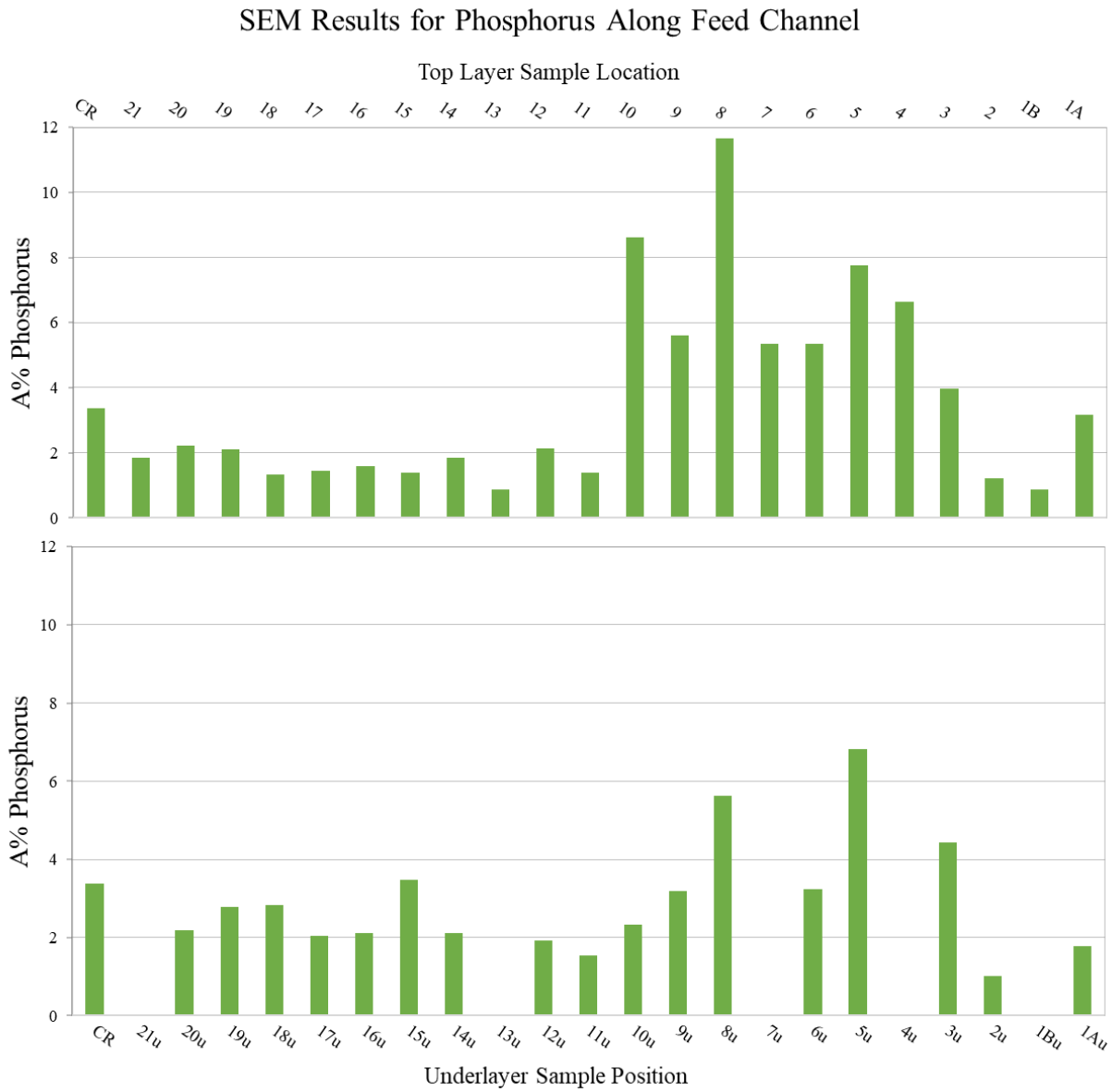

Figure 24. Atom percentages of phosphorus at each sample location

Iron and phosphorus display very similar trends in the top and bottom layers of sediment. You can clearly see their sudden increases in concentration at location 10, which is right after the wastewater inlet. This makes sense because the last step in the WWTP to remove phosphorus is not sufficient enough, so FeCl is added to precipitate out phosphorus. Therefore, the spikes in Fe and P after the wastewater inlet are expected.

The highest concentration that Fe reaches is about 9% at location 8, and the highest concentration that P reaches is about 11.7% at location 8. This implies that most of the Fe and P almost immediately settled in a short distance of roughly 100 m. The highest concentration of Fe in the underlayer is about 5.2% at location 5u, and that of P is about 6.75% at location 5u. This indicates that Fe and P percolated deeper into the sediment at a further distance from the

wastewater inlet. The concentrations gradually get smaller towards location 1 (right before the CW inlet), indicating that the rest of the Fe and P took a longer time to eventually settle into the sediment as the water flowed. The final concentration of Fe before the CW inlet is around 2%, and that of P is around 3%.

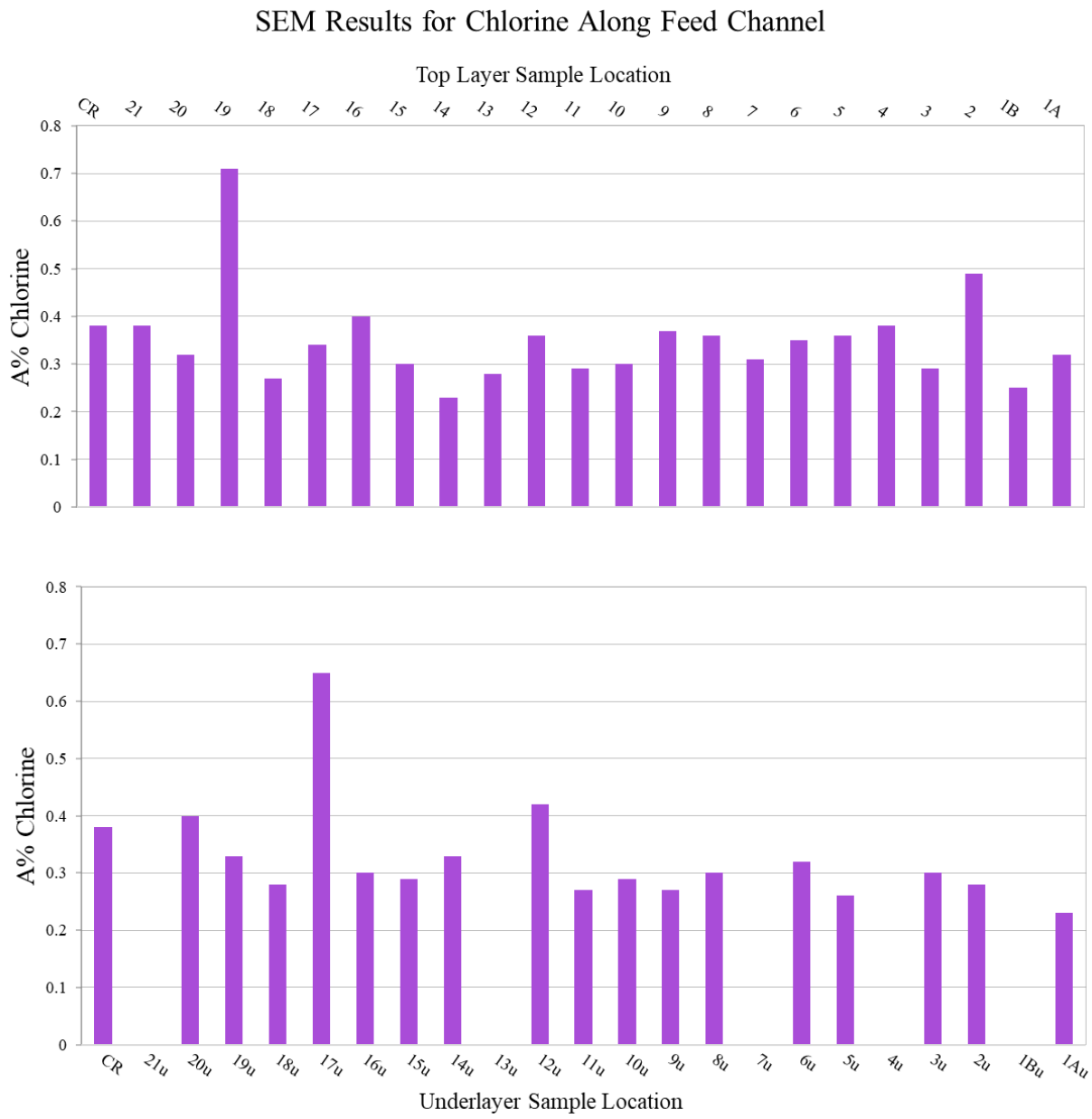

Figure 25. Atom percentages of chlorine at each sample location

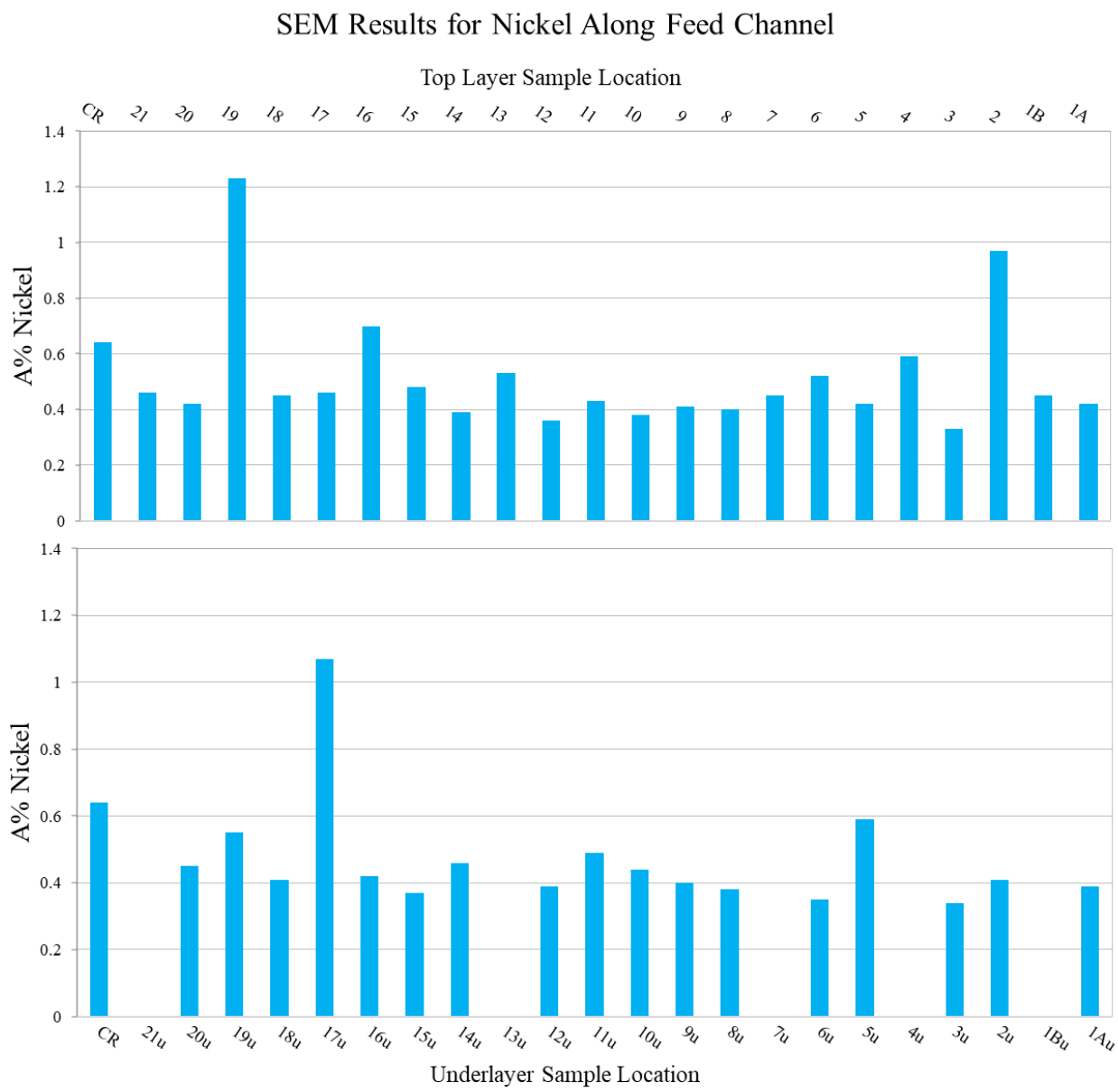

Figure 26. Atom percentages of nickel at each sample location

We also graphed results for Cl as well as Ni due to its toxicity, as shown by Figures 25 and 26 above. Even though the addition of FeCl was the last treatment step of the plant, the data for Cl does not exhibit the same trends as that for Fe and P. There is no sudden increase in concentration of Cl at location 10 like we would expect. Surprisingly, it exhibits the same trends as the data for Ni instead. The highest concentration of Cl is about 1.2% and that of Ni is about 1.06%, both of which occur at location 19, roughly 100 m after the stormwater inlet. In the underlayer, the highest concentrations occur at location 17u. The concentrations remain relatively the same with Cl under 0.4% and Ni under 0.65% until location 2 in the toplayer, where there is another increase. Both are incorporated in some particulate matter or absorbed onto particles, and the reason for the higher concentrations at certain points is unclear.

Similar trends were seen with the samples taken from the second sample collection (samples R68-1A to R68-3C). These trends are that Ca made up almost 50% of the samples, there are higher concentrations of Fe and P in samples taken downstream the wastewater inlet, and concentrations of Cl and Ni in the stormwater stayed relatively the same across the feed channel.

4.3 PAH Levels Detected by High Performance Liquid Chromatography

During the HPLC analysis, four wavelengths (205 nm, 220 nm, 254 nm, and 300 nm) were used to provoke good responses from each of the 16 PAHs. Figure 27 below shows the response of the standard PAH mix as time goes on to each of the four analytical wavelengths. Each response showed a peak at the corresponding retention time and was recorded in units of intensity. Because this is a standard solution containing only solvent and 16 PAHs, each peak corresponds to a different PAH. Each PAH shows a best response to one of the four wavelengths, as detailed in Appendix A Table A4.

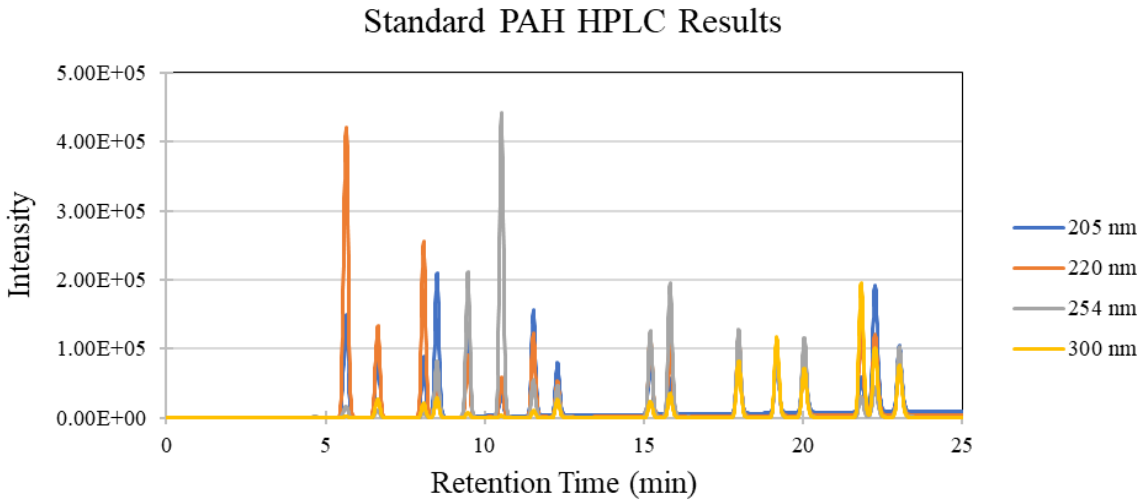

Figure 27. HPLC results for the PAH standard solution

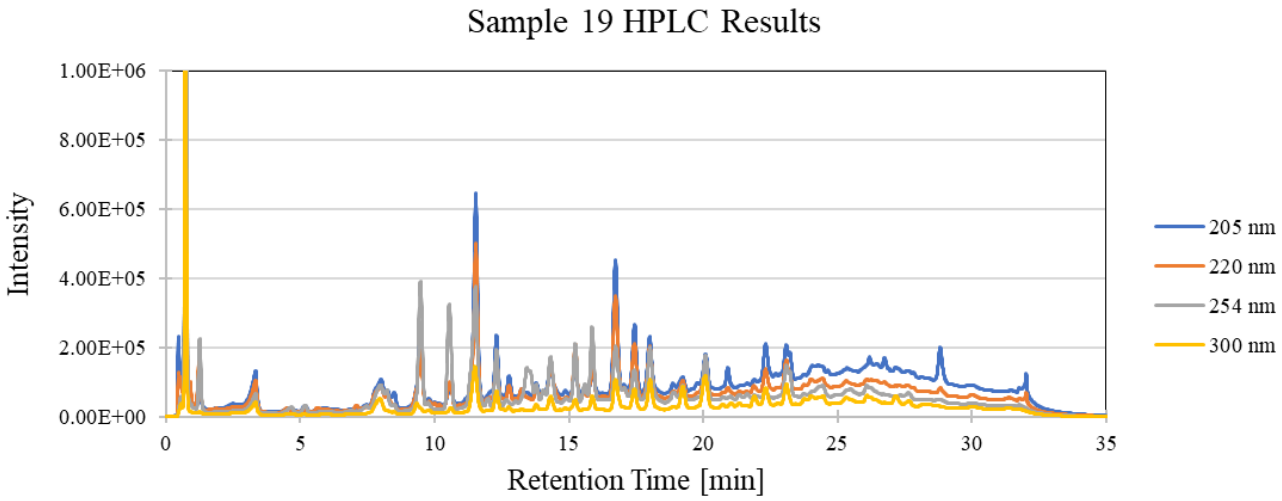

Figure 28. Example of HPLC results for a sample

Figure 28 above shows the response of sample 19 as time goes on to each of the four analytical wavelengths. Other organic material is present in the sample, as seen by the early spikes (prior to 5 minutes) and the smaller peaks throughout the sample. However, peaks representing the PAHs can still be seen at some of the corresponding retention times. Some PAHs were not detectable in all samples. For example, the standard solution showed a PAH spike at 5.65 minutes (Figure 27), and there is no visible peak at that time in the same graph for sample 19.

Two standard solutions of PAH Mix-9 were analyzed to determine which wavelength provoked the best response from each PAH. The intensities of the peaks at the best wavelength were divided by the known concentration of PAH in the standard sample, which was 10 µg/mL, to calculate a response coefficient (Appendix B, Table B2). The coefficients from both standards were averaged. Then, for each sample, the peak at each retention time was located and the intensity at the determined best wavelength was recorded. Samples 1Au, 6u, and 17 did not have enough liquid volume present after evaporation to analyze, and so are not included in this analysis. The intensities were then divided by the response coefficient to calculate the concentration (in µg/mL) of each PAH detected in each sample. If there was no peak present within ± 0.09 mins of the theoretical retention time, the PAH corresponding to that retention time was considered to be undetectable in the sample, and the concentration was recorded as zero. For each sample, the relative proportions of each PAH were graphed.

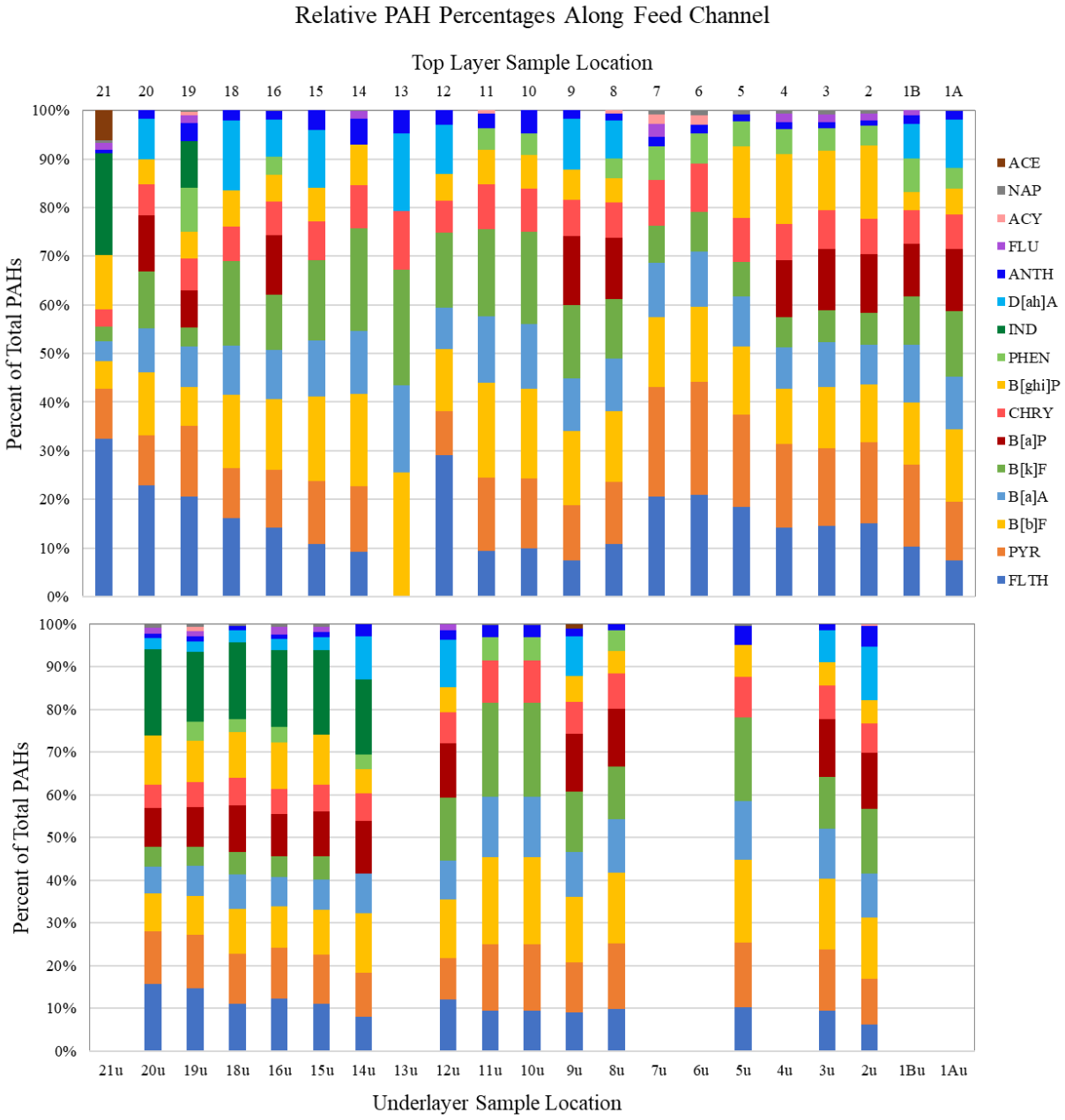

Figure 29. Relative amounts of each PAH present in each sample

The two graphs in Figure 29 above show the proportion of each PAH detected in each sample. The top graph shows these proportions for the top layer of each sample, and the bottom graph shows the proportions for the underlayer including only the samples in which the underlayer was available to analyze. The relative amounts of each PAH detected varies between samples.

In Figure 30 below, the spiked samples show a fairly even distribution of PAHs. This was expected because the PAH mixture that was added to these samples had equal concentrations of each PAH.

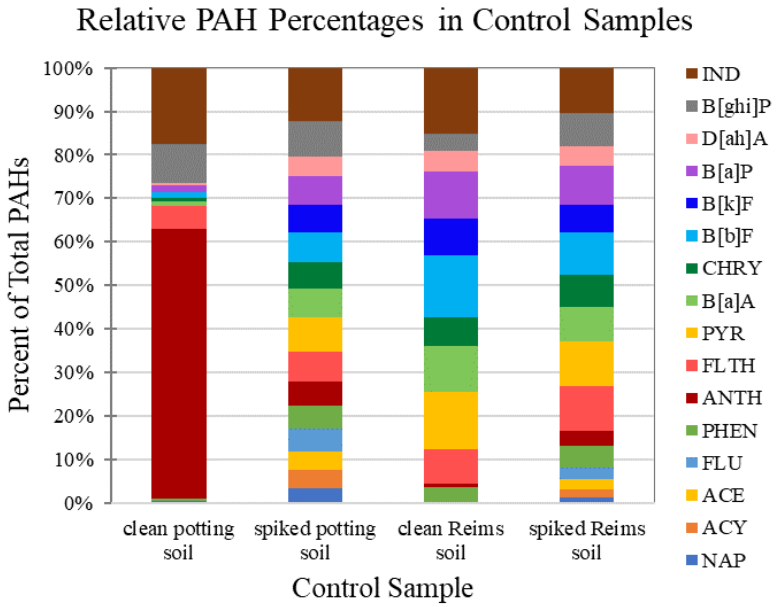

Figure 30. Relative PAH amounts detected in the spike trials

The volumes of samples analyzed by HPLC were not exactly quantified, but ranged between 2 and 2.5 mL per sample. Because of this uncertainty, a range of total PAHs detected within each sample was calculated. The concentrations of each of the 16 PAHs, calculated as described in the previous paragraph, were summed for each sample. This total concentration was then multiplied by 2 mL to obtain a low estimate of total µg of PAHs present within the sample, and also multiplied by 2.5 mL to obtain a high estimate. Each of these estimates were then divided by the mass of soil used in the extraction to determine a concentration of PAHs in each sample in units of μg PAH/g soil. These ranges were graphed, as shown in Figure 31. The top graph shows the range for the top layer of each sample, and the bottom graph shows the range for the underlayer including only the samples in which the underlayer was available to analyze.

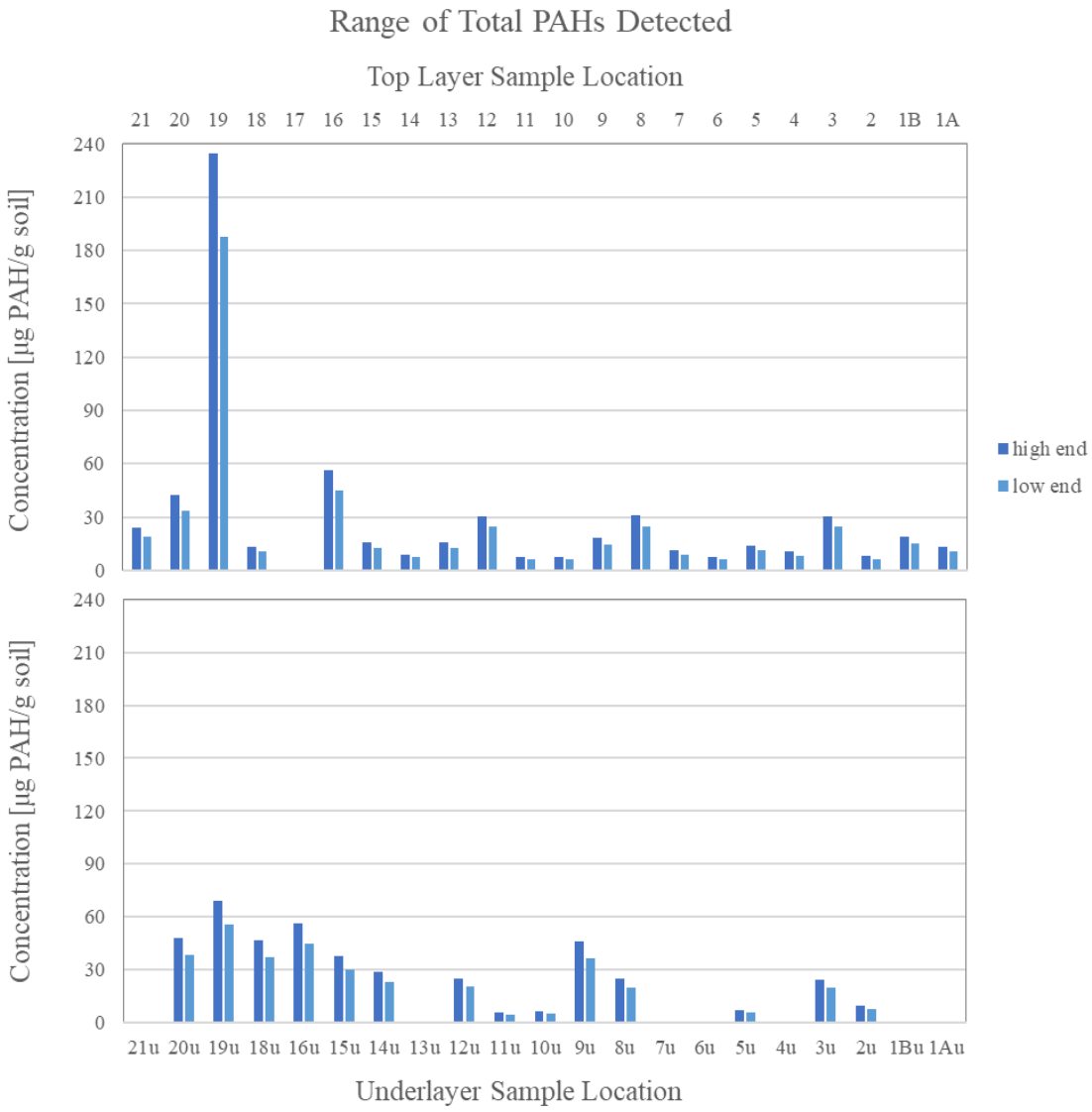

Figure 31. Range of total PAH concentration in soil

It can be observed in Figure 31 that sample 19 contained the highest concentration of total PAHs at a high estimate of 235 μm PAH/g soil in comparison to the other samples. We hypothesize that this is likely because of the presence of thick vegetation around that spot in the channel. The sediment containing PAHs after entering the channel likely flows downstream past spots 21 and 20. Then, it is caught by the vegetation and mostly settled in one place. This spike in concentration does is not as dramatic in the underlayer at a high estimate of 69 μg PAH/g soil, indicating that most PAHs settle on top of the sediment and either stay there or take some time to percolate.

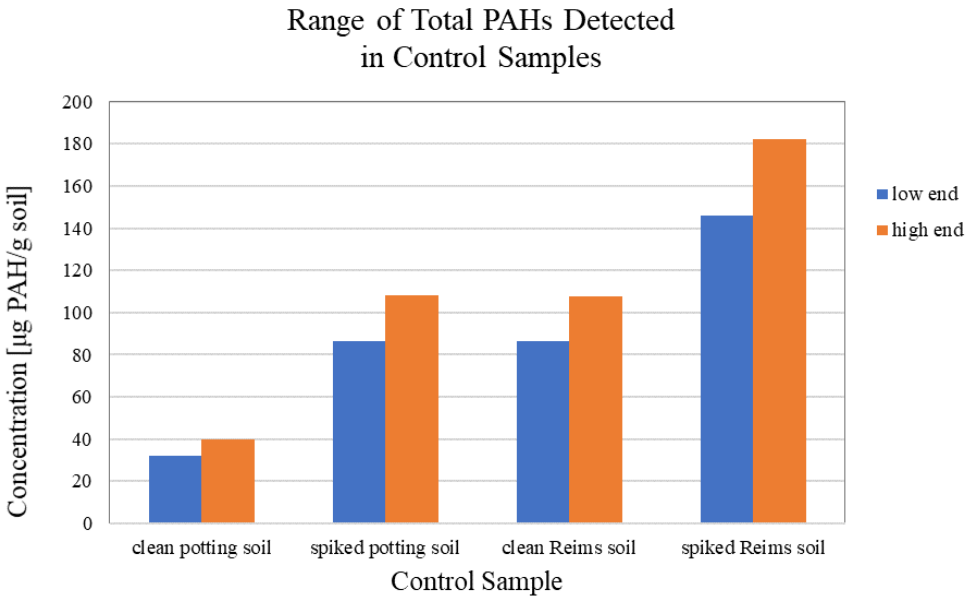

Figure 32. Concentration range of total PAHs detected in spike trials

Looking at Figure 32 above, the increase in detected PAHs after spiking confirms that the HPLC analysis accurately detects PAHs in a sample. In addition, the total PAHs present in the spiked potting soil is about equal to the total PAHs in the "clean Reims" sample, which indicates that the soil in Reims outside of the channel already contains some amount of PAHs. However, sample 19 shows a significantly higher concentration than that of the control Reims soil, which is at a high estimate of 108 μg PAH/g soil (Figure 32). Note that the "clean Reims soil" was only collected from one spot near the CW, and cannot necessarily be assumed representative of all soil in the area.

4.4 No Detection of PAHs from Near-Infrared Spectra

Functional groups have unique structures, bonds, and infrared spectra that will display multiple peaks. The pattern of peaks can help define the presence of a specific functional group in a sample. The main feature of PAHs, specifically, is their aromatic benzene rings. The aromatic bonding is what associates them with unique spectra. Ideally, peaks will be intense and easy to distinguish, will appear in a region where no other functional groups appear, and will fall in a narrow wavelength range regardless of what molecule the functional group appears in (Smith, 2016). However, the complexities of NIR spectra stemming from overlapping bands make spectra not that easy to interpret.

We obtained values of log(1/R) at wavelengths between 1000 nm and 2500 nm in intervals of 1 nm. In order to analyze the NIR spectra, first we smoothed the data by calculating the moving averages of 5 points. Then, we took the slope of every moving set of 3 points, which is denoted as "1st Derivative" in the graphs. Graphing the first derivative will allow us to identify minima and maxima of the log(1/R) values, peaks that may be characteristic of PAH compounds. Positive slopes around 1647 nm are consistent with absorptions due to vibrational stretching modes of bonds in aromatic C-H functional groups linked to PAHs (Okparanma et al.,

2013). Therefore, in our analysis, we searched for any peaks around 1647 nm that may indicate the presence of a PAH compound.

We found no peaks around 1647 nm in the spectra of any of the samples taken from AZHUREV. An example graph showing the reflectance values for the clean control samples and sample 1A are shown in Figure 33 below. It is apparent that the curves show no change in slope between 1600 and 1700 nm.

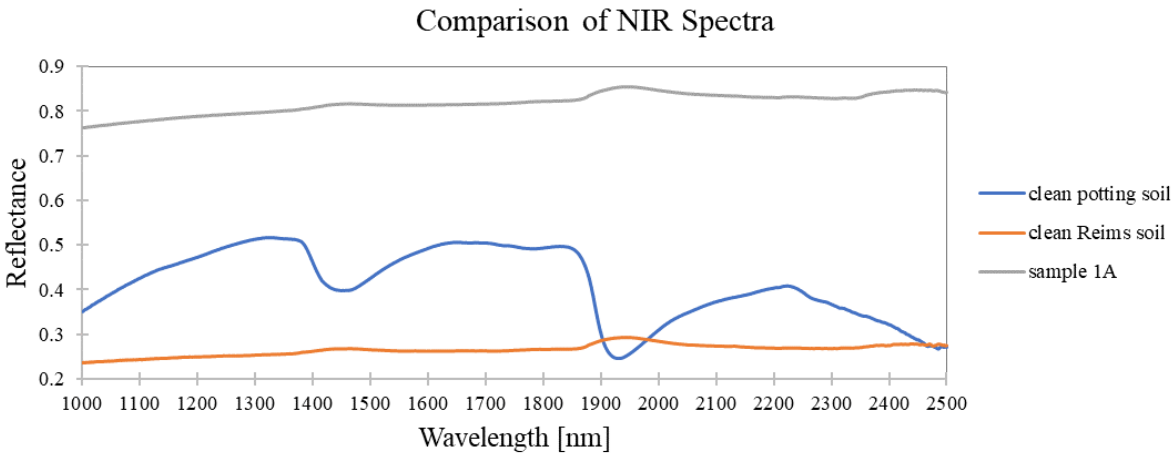

Figure 33. Reflectance values for the control samples and sample 1A at wavelengths between 1000 and 2500 nm

A closer look at the spectra for sample 1A is shown in Figure 34. The first derivative stays very close to zero between 1600 and 1700 nm, indicating that there is definitely no peak at 1647 nm. The first derivative graphs for the other samples display a similar shape and present the same conclusion.

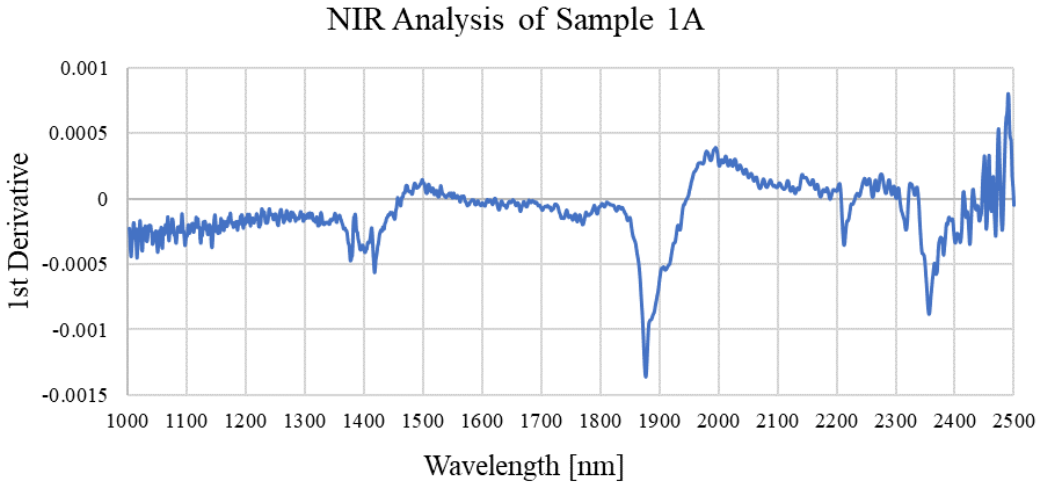

Figure 34. Changes in slope of log(1/R) values for sample 1A at wavelengths between 1000 and 2500 nm

We were unable to collect NIR spectra data for the spiked Reims and potting soil samples as well as sample 12 because there was little to no sediment left after completing ASE extractions. Therefore, without spiked sample results, we could not confirm that the NIR spectrometer was effective in detecting the presence of PAHs. If we did analyze the spiked samples and saw peaks at 1647 nm, then our results will hold more reliability.

Chapter 5: Design of PAH Removal

PAHs were detected in sediment at the AZHUREV CW site at concentrations up to 230 mg/kg. Because of the harmful environmental impacts of PAHs in soil, it is desirable to design remediation methods to lower these concentrations. What follows is a hypothetical treatment plan to remove PAHs from the sediment in the AZHUREV channel.

It should be considered that the purpose of the CW is to treat a combination of stormwater and effluent from a wastewater treatment plant before release into the nearby Vesle River. This water treatment is accomplished in part by the settling of contaminated sediments out of the streamflow, so care should be taken when designing remediation plans for the soil so as to not recontaminate the water in the process. With this consideration, the proposed remediation design avoids dredging of the sediment, as this process would stir up and reintroduce contaminated sediments into the water. Treatment methods would be applied directly to the sediment layer in the field.

In order to remove PAHs from the contaminated soil, various remediation technologies can be used. The first method is bioremediation, which is an environmentally-friendly technology that uses microorganisms to degrade organic pollutants from contaminated environments. However, there are limitations in this type of design for its application in the AZHUREV CW. It would be difficult to design a bioremediation without knowing the specific species of microorganisms already present in the water and sediment, and altering the ecology of the CW would not be ideal. Additionally, the process of bioremediation is long and can be incomplete due to low PAH availability and defiance to microorganisms (Lemaire et al., 2019).

The other method is in-situ chemical oxidation, which is the injection of an oxidant into the groundwater or soil without excavation. This remediation technique seems much easier to design for since its application is based on the organic matter and PAH content of the site (Lemaire et al., 2019). We have thoroughly analyzed these characteristics of the AZHUREV CW, so there is enough information and data to draw from. However, there are numerous obstacles using this technology. PAHs can be strongly absorbed onto organic matter or part of complex soil structures. This makes them less available and more difficult to treat. Also, a high organic content in the sediment can consume much of the oxidant, limiting the degradation of PAHs. There is a significant amount of organic matter in the AZHUREV sediment, posing a problem that may hinder oxidation efficiency. Finally, there are not enough feasibility or case studies about in-situ chemical oxidation, both at the lab scale and the field scale. This brings to light a need for more research and controlled applications before use in actual remediation sites (Leimare et al., 2019).

There are four types of oxidants that can be used for in-situ injection: permanganate, persulfate, persulfate activated with hydrogen peroxide, or Fenton's reagent. The use of different oxidants leads to a wide range of efficacies due to differences in soil characteristics such as PAH availability and distribution, pH, and organic content. Permanganate is the most effective

whatever the conditions, even if low PAH availability limits its effect, and it performs effectively over a large range of pHs (Lemaire et al., 2019). However, a major drawback is that low doses of permanganate induces the formation of toxic byproducts (Boulangé, 2019). We want to avoid further pollution of the AZHUREV CW and limit disruption of the natural habitat and wildlife, so the use of permanganate, although effective, is not the ideal choice. In our case, Fenton's reagent seems to be the best choice among the oxidants. Although it leads to lower degradation yields as compared to permanganate, it aligns well with our environmental goals and the characteristics of the wetland.

Fenton oxidation consists of the reaction of hydrogen peroxide with ferrous ions to produce ferric ions and the hydroxyl radical. This hydroxyl radical is a powerful oxidant which has been shown to be able to degrade PAHs sorbed to soil. Classical Fenton reactions use the addition of an acidic iron solution with the hydrogen peroxide in order to catalyze the production of the radicals. However, iron already present in the soil can cause the same effect. The use of endogenous iron as the catalyst for the Fenton reaction is referred to as a "Fenton-like" reaction and is more beneficial under field conditions since the addition of acidic iron solutions can change the pH of the soil and cause ecological harm (Cheng et al., 2016; Lemaire et al., 2019; Yap et al., 2011). Therefore, treatment of the AZHUREV CW will only require the addition of hydrogen peroxide since there are sufficient trace amounts of iron deposited in the sediment along the feed channel.

An important consideration for this method of removal is the amount of organic matter present in the soil. When trialing various oxidation methods for PAH remediation, a 2013 study observed that other organic matter present in the samples consumed some of the hydroxyl radical, decreasing its ability to degrade PAHs. In that study, the organic matter content of the soil used was considered high at 71.5 g/kg. In the AZHUREV channel, the observed organic matter content ranged from 25 g/kg to 450 g/kg. Because the organic content reaches higher levels, a higher initial dose of hydrogen peroxide (creating a higher ratio of hydrogen peroxide to PAH content) is desirable to allow for excess radicals to be consumed (Lemaire et al., 2019).

In addition, it has been observed that higher concentrations of hydrogen peroxide allow for increased reaction kinetics and increase the overall efficiency of PAH degradation. Furthermore, the Fenton reaction has a direct influence on microbial soil activity. When hydrogen peroxide concentrations of less than 12% were used for treatment, rebound of biological activity occurs within 6-16 weeks. Higher concentrations, however, cause biological activity to decrease for much longer periods of time. (Yap et al., 2011) Because of this ecological safety consideration, a concentration of 10% hydrogen peroxide was chosen for this hypothetical design.

With these considerations, the dosage of hydrogen peroxide depends on the amount of PAHs present in the soil, the desired ratio of hydrogen peroxide to PAH content, and the amount of soil to be treated. The highest measured concentration of PAHs in the AZHUREV channel sediment ranged from 187.7 to 234.6 mg/kg. Complete removal of PAHs has been achieved using ratios between 10:1 and 40:1 hydrogen peroxide to contaminant content. Considering the relatively high organic content in the sediment, the higher end of this dosage range should be used. A 40:1 ratio, designed for the highest possible concentration of PAHs, yields 9.38 g hydrogen peroxide/kg soil, which with 10% hydrogen peroxide becomes a dose of 93.8 mL hydrogen peroxide/kg soil.

To calculate the mass of treated soil, the channel dimensions were defined as follows. The depth of the contaminated top layer was assigned to be 2 cm. In this layer was a much higher concentration of PAHs detected. The channel is roughly 1 km long and 1 m wide. If the entire length of the channel is treated, the volume of soil is 20 m^3. Unfortunately, the density of the soil was not measured during the analytical steps of this project, so it would need to be confirmed before implementing this remediation plan. However, to roughly estimate the total amount of hydrogen peroxide needed, a density of 1300 kg/m^3 is assumed. Therefore, the total amount of hydrogen peroxide needed to treat the entire length of the channel would be 2438.8 L of 10% hydrogen peroxide. However, this amount could be greatly reduced, because the highest amount of PAHs detected was at sample location 19, and the concentration in the other samples was much less with none of the other samples exceeding 60 mg/kg PAH content. This concentration is around the same amount as was detected in the unspiked potting soil and so could be considered negligible. So, to conserve materials, only the area around sample location 19 is necessary to treat. Each location was 50 m apart, so the total volume of soil to be treated at location 19 is roughly equal to 1 cubic meter. This allows for 121.9 L of hydrogen peroxide to be used, which is a more reasonable volume.

To treat the soil, hydrogen peroxide can be injected into the sediment layer using syringes. For the 50 meter length of the channel corresponding to location 19 (beginning 25 m downstream of the original sample location and ending 25 m upstream), 2.44 L of hydrogen peroxide can be injected at every 1 meter. Because the degradation of PAHs by Fenton oxidation occurs within a few hours (Yap et al., 2011), the soil can be tested again using the methods in this paper after 1-2 days. If PAH levels are not sufficiently lowered, the process can be repeated after 16 weeks to allow for rebound of microbial activity in the soil and mitigate ecological harm.

Chapter 6: Conclusion

The analysis of the sediments along the AZHUREV channel show settling trends that match our predictions. Contaminants in the stormwater flow such as nickel, chlorine, and PAHs quickly settle upstream, likely due to a combination of low flow and filtration by vegetation. Sediment that can be attributed to the treated wastewater also settles relatively quickly after entering the channel. By the time the water enters the CW, the sediment contamination is relatively low, which indicates that most contaminants are settling out of the flow and the channel is contributing a positive effect to the overall goal of treating the water. Though these trends are promising, the data can not stand on its own and must be validated by further research. Uncertainties during sample collection and analysis should be corrected and inconsistencies eliminated wherever possible. In addition, it should be noted that these trends may be subject to change over time under varying conditions. For example, if the Grand-Reims area sees unusually heavy precipitation during a particular season, the flow in the channel may be higher and settling may not be as effective. An especially strong flow or other environmental disturbances could potentially disrupt the sediment layer and re-release contamination into the water. So, the channel should be continuously monitored and sampled, and remediation may be necessary. A couple treatment options include excavation of some contaminated sediment every few years or in-situ chemical oxidation. This project represents the beginning of an investigation into the fate and characteristics of sediment in the AZHUREV channel that should be continued and expanded upon in the future.

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Appendix A: Raw Data Tables

Table A1. Coordinates of AZHUREV Sample Stakes

Sample #	Latitude Coordinate [°N]	Longitude Coordinate [°E]
1	49.27705	3.97923
2	49.27729	3.97996
3	49.27753	3.98050
4	49.27777	3.98104
5	49.27802	3.98168
6	49.27828	3.98220
7	49.27880	3.98279
8	49.27880	3.98333
9	49.27906	3.98390
10	49.27930	3.98451
11	49.27953	3.98512
12	49.27984	3.98565
13	49.28008	3.98621
14	49.28037	3.98678
15	49.28062	3.98733
16	49.28085	3.98796
17	49.28111	3.98847
18	49.28135	3.98908
19	49.28161	3.98964
20	49.28171	3.99032
21	49.28179	3.99082
R68-1	49.277046	3.979343

R68-2	49.277542	3.9806905
R68-3	49.278664	3.982645

Table A2. Loss by Ignition Data

Sample	Mass of pot [g]	Mass of pot+sediment [g]	Mass of pot+ash [g]
clean potting soil	23.9928	24.9411	24.5238
clean Reims soil	17.5012	18.0552	17.9494
1A	23.6985	24.4670	24.4115
1Au	21.1436	21.9120	21.8660
1B	23.7208	24.5720	24.5396
2	23.8106	24.5527	24.5338
2u	23.6924	24.5993	24.5401
3	24.5895	25.4416	25.3275
3u	18.2370	19.1585	19.0106
4	18.2397	18.8105	18.7739
5	22.5172	23.2320	23.1785
5u	24.5924	25.1528	25.0969
6	24.5571	25.4600	25.4147
6u	23.7226	24.6039	24.5218
7	23.8100	24.5109	24.4678
8	23.0622	23.5953	23.4931
8u	24.5702	25.4883	25.3975
9	21.1434	22.0877	21.9801
9u	24.5921	25.5592	25.4318
10	23.6961	24.4376	24.3969
10u	23.0472	23.9982	23.9622

11	24.5582	25.4332	25.3718
11u	23.6980	24.2319	24.1854
12	23.0487	23.6276	23.5031
12u	21.8718	22.4763	22.3537
13	22.5166	23.2165	23.1693
14	21.1450	21.6844	21.6155
14u	23.8117	24.4447	24.3477
15	21.1438	21.6503	21.5448
15u	18.2384	18.8173	18.7328
16	22.5160	23.1225	22.9825
16u	23.8110	24.7644	24.5932
17	18.2386	19.0700	18.9476
17u	23.7257	24.4168	24.3513
18	23.7230	24.3770	24.3074
18u	24.5929	25.3094	25.2221
19	17.5044	18.0490	17.9133
19u	24.5575	25.4413	25.3353
20	22.5171	23.1707	23.0806
20u	23.0465	23.7719	23.6392
21	23.9839	24.6537	24.5323
R68-1A	22.5267	23.4747	23.4000
R68-1B	23.7062	24.6939	24.6224
R68-1C	18.2455	19.2273	19.1152
R68-2A	23.8189	24.8015	24.7498
R68-2B	23.7109	24.7070	24.6516
R68-2C	17.5022	18.4248	18.3662

R68-3A	20.5699	21.5526	21.5071
R68-3B	24.7708	25.6451	25.5666
R68-3C	24.1057	25.0292	24.9584

Table A3. ASE Data

Sample	Mass of vial [g]	Mass of sediment [g]	Mass of vial+solvent+extracted contaminants [g]
clean potting soil	37.3901	1.7729	58.9206
clean Reims soil	37.3901	1.8095	58.9660
spiked potting soil	37.3901	1.5962	58.6780
spiked Reims soil	37.3901	1.9333	58.6571
1A	37.5950	2.0020	N/A
1Au	37.6790	2.0925	N/A
1B	37.1351	2.5984	N/A
2	37.4159	2.9419	58.8374
2u	37.2541	3.3080	58.8577
3	37.4400	2.2644	N/A
3u	38.3457	3.0913	N/A
4	37.8458	2.4320	N/A
5	37.1085	2.5454	58.4889
5u	40.9596	2.1178	62.2787
6	39.1434	2.9730	60.5006
6u	40.5517	2.2692	N/A
7	37.1134	2.0057	58.7631
8	38.8580	2.1226	59.7015
8u	3.4014	39.7896	60.9414

9	39.5544	2.6121	59.5777
9u	39.6090	3.0329	60.2597
10	39.8180	2.6449	61.3156
10u	38.9871	2.7694	60.3441
11	41.1053	2.9107	62.7118
11u	40.7223	2.0596	62.3043
12	39.9251	2.1546	61.5542
12u	38.3034	2.1302	59.9035
13	40.8321	2.0259	N/A
14	40.4909	2.0604	N/A
14u	40.9913	2.7670	N/A
15	37.8388	2.6020	59.4115
15u	37.5300	2.2568	59.3553
16	40.6915	2.5065	N/A
16u	40.5261	2.4185	N/A
17	40.5595	2.2064	62.3561
17u	40.9165	2.8379	N/A
18	37.8797	2.4665	59.6763
18u	37.5387	2.4820	59.2790
19	37.9291	2.1238	59.6075
19u	37.4591	3.1344	59.5548
20	37.6483	2.5142	59.1567
20u	38.0346	2.5757	59.0707
21	37.2635	2.1414	58.5624

Note. The masses of the vials for the control and spiked samples were assumed to be 37.3901 g since they were not individually measured before the ASE. Masses labeled N/A were not measured; either possible errors occurred during the extraction or vial filters had to be changed.

Table A4. PAH HPLC Standards

PAH	Retention Time [min]	Best Wavelength [nm]
NAP	5.65	220
ACY	6.65	220
ACE	8.1	220
FLU	8.5	205
PHEN	9.5	254
ANTH	10.5	354
FLTH	11.5	205
PYR	12.3	205
B[a]A	15.2	254
CHRY	15.8	254
B[b]F	18	254
B[k]F	19.2	300
B[a]P	20	254
D[ah]A	21.8	300
B[ghi]P	22.25	205
IND	23	205

Appendix B: Calculations

Table B1. Determining the Concentration of Organic Matter in Sediment Samples

Sample	Mass of sediment [g]	Mass of OM [g]	Concentration of OM in sediment [mg/g]	LOI (% wt)
clean potting soil	0.9483	0.4173	440.1	44.01
clean Reims soil	0.5540	0.1058	191.0	19.10
1A	0.7685	0.0555	72.22	7.222
1Au	0.7684	0.0460	59.87	5.987
1B	0.8512	0.0324	38.06	3.806
2	0.7421	0.0189	25.47	2.547
2u	0.9069	0.0592	65.28	6.528
3	0.8521	0.1141	133.9	13.39
3u	0.9215	0.1479	160.5	16.05
4	0.5708	0.0366	64.12	6.412
5	0.7148	0.0535	74.85	7.485
5u	0.5604	0.0559	99.75	9.975
6	0.9029	0.0453	50.17	5.017
6u	0.8813	0.0821	93.16	9.316
7	0.7009	0.0431	61.49	6.149
8	0.5331	0.1022	191.7	19.17
8u	0.9181	0.0908	98.90	9.890
9	0.9443	0.1076	113.9	11.39
9u	0.9671	0.1274	131.7	13.17
10	0.7415	0.0407	54.89	5.489
10u	0.9510	0.0360	37.86	3.786
11	0.8750	0.0614	70.17	7.017

11u	0.5339	0.0465	87.10	8.710
12	0.5789	0.1245	215.1	21.51
12u	0.6045	0.1226	202.8	20.28
13	0.6999	0.0472	67.44	6.744
14	0.5394	0.0689	127.7	12.77
14u	0.6330	0.0970	153.2	15.32
15	0.5065	0.1055	208.3	20.83
15u	0.5789	0.0845	146.0	14.60
16	0.6065	0.1400	230.8	23.08
16u	0.9534	0.1712	179.6	17.96
17	0.8314	0.1224	147.2	14.72
17u	0.6911	0.0655	94.78	9.478
18	0.6540	0.0696	106.4	10.64
18u	0.7165	0.0873	121.8	12.18
19	0.5446	0.1357	249.2	24.92
19u	0.8838	0.1060	119.9	11.99
20	0.6536	0.0901	137.9	13.79
20u	0.7254	0.1327	182.9	18.29
21	0.6698	0.1214	181.2	18.12

Table B2. Calculation of HPLC Response Coefficients

PAH	Peak Intensity (Standard 1)	Response Coefficient (Standard 1)	Peak Intensity (Standard 2)	Response Coefficient (Standard 2)	Average Response Coefficient
NAP	420179	42017.9	434132	43413.2	42715.55
ACY	132740	13274	137865	13786.5	13530.25
ACE	255464	25546.4	263311	26331.1	25938.75

FLU	210339	21033.9	216783	21678.3	21356.1
PHEN	212264	21226.4	218015	21801.5	21513.95
ANTH	441987	44198.7	450864	45086.4	44642.55
FLTH	155910	15591	158412	15841.2	15716.1
PYR	80870	8087	82042	8204.2	8145.6
B[a]A	125997	12599.7	127016	12701.6	12650.65
CHRY	195511	19551.1	196793	19679.3	19615.2
B[b]F	127232	12723.2	128360	12836	12779.6
B[k]F	116748	11674.8	117814	11781.4	11728.1
B[a]P	116044	11604.4	116216	11621.6	11613
D[ah]A	194773	19477.3	196922	19692.2	19584.75
B[ghi]P	192126	19212.6	197746	19774.6	19493.6
IND	105279	10527.9	112848	11284.8	10906.35

Note. Each PAH concentration in the standard equals 10 µg/mL.

Sample Calculations

The following are sample calculations using the clean potting soil sample data.

Concentration of Organic Matter in Sediment

Mass of sediment = (mass of pot+sediment) - (mass of pot)

= (24.9411 - 23.9928) g = 0.9483 g

Mass of organic matter = (mass of pot+ash) - (mass of pot+sediment)

= (24.5238 - 24.9411) g = 0.4173 g

Concentration of OM = $\frac{mass\ of\ OM}{mass\ of\ sediment} = \frac{0.4173\ g}{0.9483\ g} \cdot \frac{100\ mg}{g} = 440.1\ mg/g$

Loss on Ignition

$$LOI = \frac{(mass\ of\ pot+sediment)-(mass\ of\ pot+ash)}{mass\ of\ sediment} \cdot 100\% = \frac{(24.9411-24.5238)\ g}{0.9483\ g} = 44.01\%$$

HPLC Response Coefficients

Response Coefficient = $\frac{Peak\ Height\ (intensity)}{Known\ PAH\ concentration\ (\mu g/mL)} = \frac{420179}{10} = 42017.9$

HPLC Quantifications

Concentration PAH in extracted solvent (µg/mL) $= \frac{Peak\ Height\ (intensity)}{Average\ Response\ Coefficient} = \frac{5539}{42715.55}$ = 0.1297 µg/mL

Concentration total PAH in soil sample (low estimate) (µg/g) = $\frac{(\sum PAH\ concentration\ in\ extracted\ solvent\ (\mu g/mL) \cdot 2\ mL)}{Mass\ of\ extracted\ soil\ (g)} = \frac{(10.74559018\ \mu g/mL \cdot 2\ mL)}{2.002\ g}$ = 10.735 µg/g

Concentration total PAH in soil sample (high estimate) (µg/g) = $\frac{(\sum PAH\ concentration\ in\ extracted\ solvent\ (\mu g/mL) \cdot 2.5\ mL)}{Mass\ of\ extracted\ soil\ (g)} = \frac{(10.74559018\ \mu g/mL \cdot 2.5\ mL)}{2.002\ g}$ = 13.419 µg/g

Appendix C: Information on PAH Mix-9

The PAH mixture used to spike the control samples is the Dr. Ehrenstorfer GmbH PAH-Mix 9 in 10 ug/mL of cyclohexane solvent. The mixture contains the 16 priority PAHs in equal concentrations as identified by the US-EPA. The following pages are the safety data sheet for the mixture (LGC Group, n.d.).

SECTION 1: Identification of the substance/mixture and of the company/undertaking

· ***1.1 Product identifier***

· ***Product name:*** **PAH-Mix 9 10 µg/mL in Cyclohexane**

· ***Part number:*** *DRE-LS20950009CY*

· ***1.2 Relevant identified uses of the substance or mixture and uses advised against***
No further relevant information available.

· ***Application of the substance / the mixture*** *Reference material for laboratory use only*

· ***1.3 Details of the supplier of the safety data sheet***

· ***Manufacturer/Supplier:***

LGC Limited
Queens Road
Teddington
Middlesex TW11 0LY
UNITED KINGDOM

Tel :+44 (0) 20 8943 7000
Fax :+44 (0) 20 8943 2767
eMail : gb@lgcstandards.com
Web : www.lgcstandards.com

· ***Further information obtainable from:***
Product safety department
eMail : sds-request@lgcgroup.com

· ***1.4 Emergency telephone number:*** *+44 (0) 20 8943 7000 (Monday - Friday : 8am - 5pm)*

SECTION 2: Hazards identification

· **2.1 Classification of the substance or mixture**
· **Classification according to Regulation (EC) No 1272/2008**

GHS02 flame

Flam. Liq. 2 H225 Highly flammable liquid and vapour.

GHS08 health hazard

Asp. Tox. 1 H304 May be fatal if swallowed and enters airways.

GHS09 environment

Aquatic Acute 1 H400 Very toxic to aquatic life.
Aquatic Chronic 1 H410 Very toxic to aquatic life with long lasting effects.

GHS07

Skin Irrit. 2 H315 Causes skin irritation.
STOT SE 3 H336 May cause drowsiness or dizziness.

· **2.2 Label elements**
· **Labelling according to Regulation (EC) No 1272/2008**
The product is classified and labelled according to the CLP regulation.

(Contd. on page 2)

(Contd. from page 1)

· **Hazard pictograms**

GHS02 GHS07 GHS08 GHS09

· **Signal word** Danger

· **Hazard-determining components of labelling:**
Cyclohexane

· **Hazard statements**
H225 Highly flammable liquid and vapour.
H315 Causes skin irritation.
H336 May cause drowsiness or dizziness.
H304 May be fatal if swallowed and enters airways.
H410 Very toxic to aquatic life with long lasting effects.

· **Precautionary statements**

P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/ doctor.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P405	Store locked up.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

· **2.3 Other hazards**
· **Results of PBT and vPvB assessment**
· **PBT:** Not applicable.
· **vPvB:** Not applicable.

SECTION 3: Composition/information on ingredients

· **3.2 Chemical characterisation: Mixtures**
· **Description:** Also contains substances at levels not considered to be hazardous.

· **Dangerous components:**

CAS: 110-82-7 EINECS: 203-806-2 RTECS: GU 6300000	Cyclohexane Flam. Liq. 2, H225; Asp. Tox. 1, H304; Aquatic Acute 1, H400; Aquatic Chronic 1, H410; Skin Irrit. 2, H315; STOT SE 3, H336	>99%
CAS: 56-55-3 EINECS: 200-280-6 RTECS: CV9275000	Benzo[a]anthracene Carc. 1B, H350; Aquatic Acute 1, H400; Aquatic Chronic 1, H410	<0.1%
CAS: 50-32-8 EINECS: 200-028-5 RTECS: DJ 3675000	Benzo[a]pyrene Muta. 1B, H340; Carc. 1B, H350; Repr. 1B, H360FD; Aquatic Acute 1, H400; Aquatic Chronic 1, H410; Skin Sens. 1, H317	<0.1%
CAS: 53-70-3 EINECS: 200-181-8 RTECS: HN 2625000	Dibenzo[a,h]anthracene Carc. 1B, H350; Aquatic Acute 1, H400; Aquatic Chronic 1, H410	<0.1%

· **Additional information:** For the wording of the listed hazard phrases refer to section 16.

SECTION 4: First aid measures

· **4.1 Description of first aid measures**
· **After inhalation:** In case of unconsciousness place patient in recovery position for transport.
· **After skin contact:**
Immediately wash with water and soap and rinse thoroughly.
If skin irritation continues, consult a doctor.
· **After eye contact:** Rinse opened eye for several minutes under running water.
· **After swallowing:**
Rinse mouth. Do not induce vomiting.
Call for a doctor immediately.
· **4.2 Most important symptoms and effects, both acute and delayed** No further relevant information available.
· **4.3 Indication of any immediate medical attention and special treatment needed**
No further relevant information available.

SECTION 5: Firefighting measures

· **5.1 Extinguishing media**
· **Suitable extinguishing agents:**
CO_2, powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
· **For safety reasons unsuitable extinguishing agents:** Water with full jet
· **5.2 Special hazards arising from the substance or mixture**
Formation of toxic gases is possible during heating or in case of fire.
· **5.3 Advice for firefighters**
· **Protective equipment:** Wear self-contained respiratory protective device.

SECTION 6: Accidental release measures

· **6.1 Personal precautions, protective equipment and emergency procedures**
Wear protective equipment. Keep unprotected persons away.
· **6.2 Environmental precautions:**
Inform respective authorities in case of seepage into water course or sewage system.
Do not allow to enter sewers/ surface or ground water.
· **6.3 Methods and material for containment and cleaning up:**
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Dispose of contaminated material as waste according to item 13.
Ensure adequate ventilation.
· **6.4 Reference to other sections**
See Section 7 for information on safe handling.
See Section 8 for information on personal protection equipment.
See Section 13 for disposal information.

SECTION 7: Handling and storage

· **7.1 Precautions for safe handling** Store in cool, dry place in tightly closed receptacles.
· **Information about fire - and explosion protection:**
Keep ignition sources away - Do not smoke.
Protect against electrostatic charges.

· **7.2 Conditions for safe storage, including any incompatibilities**
· **Storage:**
· **Requirements to be met by storerooms and receptacles:**
Please refer to the manufacturer's certificate for specific storage and transport temperature conditions.

(Contd. on page 4)

(Contd. from page 3)

Store only in the original receptacle.
Keep container in a well-ventilated place. Keep away from sources of ignition and heat.
· **Information about storage in one common storage facility:** Store away from foodstuffs.
· **Further information about storage conditions:**
Keep container tightly sealed.
Store in cool, dry conditions in well sealed receptacles.
· **7.3 Specific end use(s)** No further relevant information available.

SECTION 8: Exposure controls/personal protection

· **Additional information about design of technical facilities:** No further data; see item 7.

· **8.1 Control parameters**

· **Ingredients with limit values that require monitoring at the workplace:**	
110-82-7 Cyclohexane	
WEL	Short-term value: 1050 mg/m³, 300 ppm Long-term value: 350 mg/m³, 100 ppm

· **Additional information:** Lists used were valid at the time of SDS preparation.

· **8.2 Exposure controls**
· **Personal protective equipment:**
· **General protective and hygienic measures:**
Keep away from foodstuffs, beverages and feed.
Immediately remove all soiled and contaminated clothing
Wash hands before breaks and at the end of work.
Avoid contact with the skin.
Avoid contact with the eyes and skin.
· **Respiratory protection:**
Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced
· **Protection of hands:**
The glove material has to be impermeable and resistant to the product/ the substance/ the preparation.
Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation
The protective gloves to be used must comply with the specifications of EC Directive 89/686/EEC and the related standard EN374

Protective gloves

· **Material of gloves** Nitrile rubber, NBR
· **Penetration time of glove material**
The exact break through time has to be found out by the manufacturer of the protective gloves and has to be observed.
· **Eye protection:**

Tightly sealed goggles

SECTION 9: Physical and chemical properties

· **9.1 Information on basic physical and chemical properties**	
· **General Information**	
· **Appearance:**	
Form:	Liquid
Colour:	Colourless
· **Odour:**	Sweetish
· **Odour threshold:**	Not determined.
· **pH-value:**	Not determined.
· **Change in condition**	
Melting point/freezing point:	6.4 °C
Initial boiling point and boiling range:	81 °C
· **Flash point:**	-18 °C
· **Flammability (solid, gas):**	Not determined.
· **Ignition temperature:**	260 °C
· **Decomposition temperature:**	Not determined.
· **Auto-ignition temperature:**	Product is not selfigniting.
· **Explosive properties:**	Product is not explosive. However, formation of explosive air/ vapour mixtures is possible.
· **Explosion limits:**	
Lower:	1.2 Vol %
Upper:	8.3 Vol %
· **Vapour pressure at 20 °C:**	104 hPa
· **Density at 20 °C:**	0.78 g/cm³
· **Relative density**	Not determined.
· **Vapour density**	Not determined.
· **Evaporation rate**	Not determined.
· **Solubility in / Miscibility with water at 20 °C:**	0.05 g/l
· **Partition coefficient: n-octanol/water:**	Not determined.
· **Viscosity:**	
Dynamic at 20 °C:	0.94 mPas
Kinematic:	Not determined.
· **9.2 Other information**	No further relevant information available.

SECTION 10: Stability and reactivity

· **10.1 Reactivity** Stable under normal conditions.
· **10.2 Chemical stability** Stable under normal conditions.
· **Thermal decomposition / conditions to be avoided:**
Formation of toxic gases is possible during heating or in case of fire.
· **10.3 Possibility of hazardous reactions** May form flammable/explosive vapour-air mixture.
· **10.4 Conditions to avoid**
Sources of ignition
Heat.
· **10.5 Incompatible materials:** Strong oxidizing agents.

(Contd. on page 6)

(Contd. from page 5)

· **10.6 Hazardous decomposition products:**
Formation of toxic gases is possible during heating or in case of fire.

SECTION 11: Toxicological information

· **11.1 Information on toxicological effects**
· **Acute toxicity** Based on available data, the classification criteria are not met.

· **LD/LC50 values relevant for classification:**		
110-82-7 Cyclohexane		
Oral	LD50	12,705 mg/kg (rat)

· **Primary irritant effect:**
· **Skin corrosion/irritation**
Causes skin irritation.
· **Serious eye damage/irritation** Based on available data, the classification criteria are not met.
· **Respiratory or skin sensitisation** Based on available data, the classification criteria are not met.
· **CMR effects (carcinogenity, mutagenicity and toxicity for reproduction)**
· **Germ cell mutagenicity** Based on available data, the classification criteria are not met.
· **Carcinogenicity** Based on available data, the classification criteria are not met.
· **Reproductive toxicity** Based on available data, the classification criteria are not met.
· **STOT-single exposure**
May cause drowsiness or dizziness.
· **STOT-repeated exposure** Based on available data, the classification criteria are not met.
· **Aspiration hazard**
May be fatal if swallowed and enters airways.

SECTION 12: Ecological information

· **12.1 Toxicity**

· **Aquatic toxicity:**	
110-82-7 Cyclohexane	
LC50/96 h	42.3 mg/l (fish)
191-24-2 Benzo[ghi]perylene	
LC50/48	0.000587 mg/l (crustacean)

· **12.2 Persistence and degradability** No further relevant information available.
· **12.3 Bioaccumulative potential** No further relevant information available.
· **12.4 Mobility in soil** No further relevant information available.
· **Ecotoxicological effects:**
· **Remark:** Very toxic for fish
· **Additional ecological information:**
· **General notes:**
Water hazard class 2 (German Regulation) (Self-assessment): hazardous for water
Do not allow product to reach ground water, water course or sewage system.
Danger to drinking water if even small quantities leak into the ground.
Also poisonous for fish and plankton in water bodies.
Very toxic for aquatic organisms
· **12.5 Results of PBT and vPvB assessment**
· **PBT:** Not applicable.
· **vPvB:** Not applicable.
· **12.6 Other adverse effects** No further relevant information available.

SECTION 13: Disposal considerations

· **13.1 Waste treatment methods**
· **Recommendation**
Must not be disposed of together with household garbage. Do not allow product to reach sewage system.
· **European waste catalogue**
Waste disposal key numbers from EWC have to be assigned depending on origin and processing.

· **Uncleaned packaging:**
· **Recommendation:** Dispose of in accordance with national regulations.

SECTION 14: Transport information

· **14.1 UN-Number**	
· **ADR, IMDG, IATA**	UN1145
· **ADR**	1145 CYCLOHEXANE mixture, ENVIRONMENTALLY HAZARDOUS
· **IMDG**	CYCLOHEXANE mixture, MARINE POLLUTANT
· **IATA**	CYCLOHEXANE mixture
· **14.3 Transport hazard class(es)**	
· **ADR, IMDG**	
· **Class**	3 Flammable liquids.
· **Label**	3
· **IATA**	
· **Class**	3 Flammable liquids.
· **Label**	3
· **14.4 Packing group**	
· **ADR, IMDG, IATA**	II
· **14.5 Environmental hazards:**	Product contains environmentally hazardous substances: Cyclohexane
· **Marine pollutant:**	Symbol (fish and tree)
· **Special marking (ADR):**	Symbol (fish and tree)
· **14.6 Special precautions for user**	Warning: Flammable liquids.
· **Danger code (Kemler):**	33
· **EMS Number:**	F-E,S-D
· **Stowage Category**	E
· **14.7 Transport in bulk according to Annex II of Marpol and the IBC Code**	Not applicable.

(Contd. on page 8)

(Contd. from page 7)

· **Transport/Additional information:**

· **ADR**	
· **Limited quantities (LQ)**	1L
· **Excepted quantities (EQ)**	Code: E2 Maximum net quantity per inner packaging: 30 ml Maximum net quantity per outer packaging: 500 ml
· **Transport category**	2
· **Tunnel restriction code**	D/E
· **UN "Model Regulation":**	UN 1145 CYCLOHEXANE MIXTURE, 3, II, ENVIRONMENTALLY HAZARDOUS

SECTION 15: Regulatory information

· **15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture**

· **Philippines Inventory of Chemicals and Chemical Substances**	
110-82-7	Cyclohexane
208-96-8	Acenaphthylene
120-12-7	Anthracene
50-32-8	Benzo[a]pyrene
86-73-7	Fluorene
91-20-3	Naphthalene
85-01-8	Phenanthrene
129-00-0	Pyrene
83-32-9	Acenaphthene

· **Australian Inventory of Chemical Substances**	
110-82-7	Cyclohexane
120-12-7	Anthracene
218-01-9	Chrysene
206-44-0	Fluoranthene
86-73-7	Fluorene
91-20-3	Naphthalene
85-01-8	Phenanthrene
129-00-0	Pyrene
83-32-9	Acenaphthene

· **Standard for the Uniform Scheduling of Medicines and Poisons**		
91-20-3	Naphthalene	S6

· **Directive 2012/18/EU**
· **Named dangerous substances - ANNEX I** None of the ingredients is listed.
· **Seveso category**
E1 Hazardous to the Aquatic Environment
P5c FLAMMABLE LIQUIDS
· **Qualifying quantity (tonnes) for the application of lower-tier requirements** 100 t
· **Qualifying quantity (tonnes) for the application of upper-tier requirements** 200 t
· **REGULATION (EC) No 1907/2006 ANNEX XVII** Conditions of restriction: 3, 57

(Contd. on page 9)

(Contd. from page 8)

· ***15.2 Chemical safety assessment:*** *A Chemical Safety Assessment has not been carried out.*

SECTION 16: Other information

The information in this safety data sheet (SDS) has been prepared with due care and is true and accurate to the best of our knowledge. The user must determine the suitability of the information for its particular purpose, ensure compliance with existing laws and regulations, and be aware that other or additional safety or performance considerations may arise when using, handling and/ or storing the material. The information in this SDS does not purport to be all inclusive or a guarantee as to the properties of the material supplied, and should be used only as a guide. LGC makes no warranties or representations as to the accuracy and completeness of the information contained herein, shall not be held responsible for the suitability of this information for the user's intended purposes or the consequences of such use, and shall not be liable for any damage or loss, howsoever arising, direct or otherwise.

· ***Abbreviations and acronyms:***
ADR: Accord européen sur le transport des marchandises dangereuses par Route (European Agreement concerning the International Carriage of Dangerous Goods by Road)
IMDG: International Maritime Code for Dangerous Goods
IATA: International Air Transport Association
GHS: Globally Harmonised System of Classification and Labelling of Chemicals
EINECS: European Inventory of Existing Commercial Chemical Substances
ELINCS: European List of Notified Chemical Substances
CAS: Chemical Abstracts Service (division of the American Chemical Society)
LC50: Lethal concentration, 50 percent
LD50: Lethal dose, 50 percent
PBT: Persistent, Bioaccumulative and Toxic
vPvB: very Persistent and very Bioaccumulative
Flam. Liq. 2: Flammable liquids – Category 2
Skin Irrit. 2: Skin corrosion/irritation – Category 2
Skin Sens. 1: Skin sensitisation – Category 1
Muta. 1B: Germ cell mutagenicity – Category 1B
Carc. 1B: Carcinogenicity – Category 1B
Repr. 1B: Reproductive toxicity – Category 1B
STOT SE 3: Specific target organ toxicity (single exposure) – Category 3
Asp. Tox. 1: Aspiration hazard – Category 1
Aquatic Acute 1: Hazardous to the aquatic environment - acute aquatic hazard – Category 1
Aquatic Chronic 1: Hazardous to the aquatic environment - long-term aquatic hazard – Category 1

· ***Sources***
Tables 3.1 and 3.2 from Annex 6 of EC 1272/2008, EC 1907/2006, EH40/2005 as amended 2011, Registry of Toxic Effects of Chemical Substances (RTECS), The Dictionary of Substances and their Effects, 1st Edition, IUCLID.

· ***Data compared to the previous version altered.*** *All sections have been updated.*